Thesis for the Master's Degree in Chemistry

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Synthesis of 9-Substituted-8-oxoadenines as Potential Inhibitors of DNA Repair Enzymes in Cancer Therapy

60 study points

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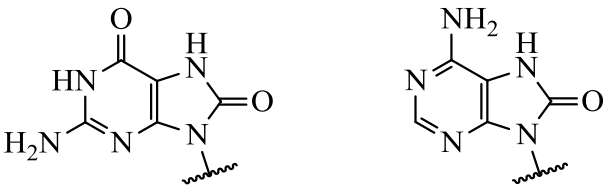
ABSTRACT

Cancer is a well-known group of diseases with a plethora of available treatments that have varying side-effects and patient survival rates. One aspect of cancer treatment that has not been investigated in depth is the possibility of inhibiting enzymatic cell repair to increase cancer cell death and dormancy due to chemotherapy and radiation therapy.

The 7,8-dihydro-8-oxoguanine (**1**) and the 7,8-dihydro-8-oxoadenine (**2**) lesions (shown below in Figure 1) are modified DNA bases that have been oxidised by reactive oxygen species (ROS), for example through cancer treatment, and have been identified has having high mutagenic potential and therefore suitable to cause dormancy or death of cancer cells.[1-3]



1 **2**

Figure 1. 7,8-dihydro-8-oxoguanine (**1**) and the 7,8-dihydro-8-oxoadenine (**2**) lesions, attached at *N*-9 to the sugar and phosphate backbone in DNA.

This project has focused on the synthesis of 9-substiuted-8-oxoadenines (**3**) as potential inhibitors (Figure 2), and this has required the use of several different strategies. In addition, some attention has been given to the possibilities of functionalising the C-8 position on the purine ring as part on an ongoing project in the Gundersen group at UiO.

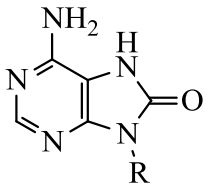


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Figure 2. General structure of target molecules.

ABBREVIATIONS

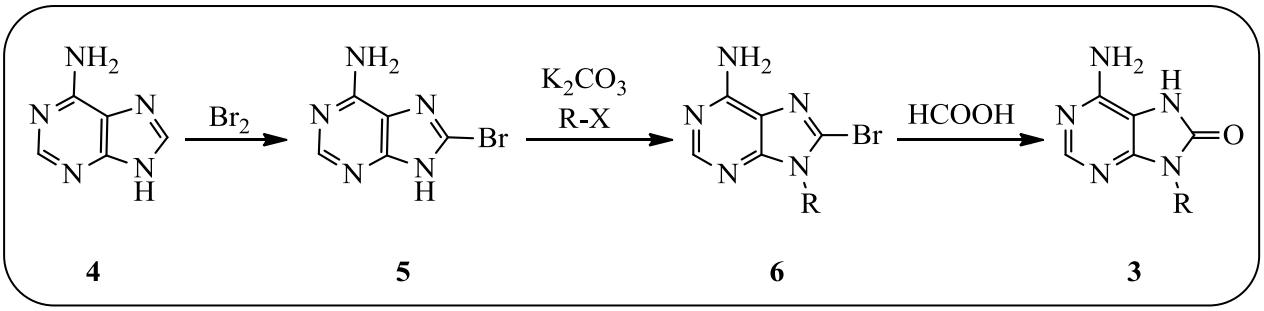
Ac	Acetyl
AGT	O^6-Alkylguanine DNA Methyltransferase
APE1	Apurinic endonuclease 1
Ar	Aromatic
ATM	Ataxia Telangiectasia
BER	Base Excision Repair
BG	O^6-Benzylguanine
Bn	Benzyl
br	Broad (NMR)
CDI	Carbonyldiimidazole
conc.	Concentrated
d	Doublet (NMR)
DCM	Dichloromethane
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
E	Electrophile
eq	Equivalents
ESI-MS	Electrospray-Ionisation Mass Spectrometry
Et	Ethyl
exp.	Experimental
hAAG	Human Alkyladenine DNA Glycosylase
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
hOGG1	Human 8-Hydroxyguanine DNA Glycosylase
HR-MS	High-Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IUPAC	International Union of Pure and Applied Chemistry
L	Ligand
LDA	Lithium Diisopropylamide
lit.	Literature

Me	Methyl
MMR	Mismatch Repair
M.p.	Melting point
MS	Mass Spectrometry
N/A	Not Applicable
n-Bu	*n*-Butyl
NBS	*N*-Bromosuccinimide
NER	Nucleotide Excision Repair
NHEJ	Non-Homologous End-Joining
NMR	Nuclear Magnetic Resonance
NOE(SY)	Nuclear Overhauser Effect (Spectroscopy)
Nu	Nucleophile
p	Pentet (NMR)
Ph	Phenyl
PARP	Poly (ADP) Ribose Polymerase
PMBA	*para*-Methoxybenzylamine
Ref-1	Redox factor-1
R_f	Retention Factor
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rel. int.	Relative Intensity
r.t.	Room Temperature
s	Singlet (NMR)
SAR	Structure Activity Relationship
S.M.	Starting Material
S_NAr	Nucleophilic Aromatic Substitution
t	Triplet (NMR)
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatography
UiO	Universitetet i Oslo (The University of Oslo)
WHO	World Health Organisation

CONTENTS

GRAPHICAL ABSTRACTS

Strategy 1:



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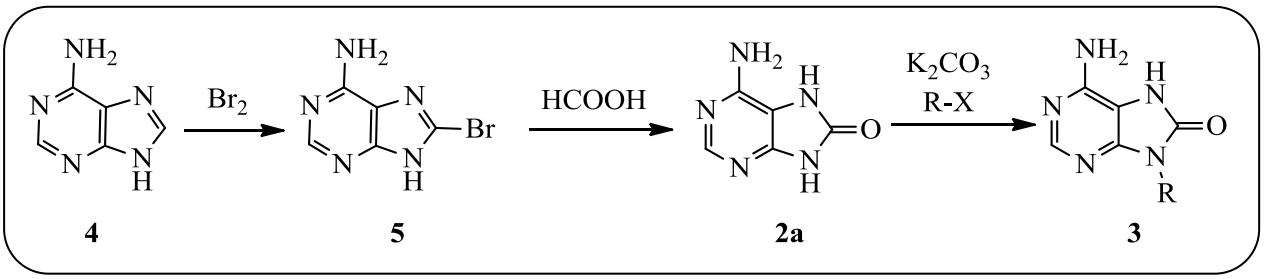

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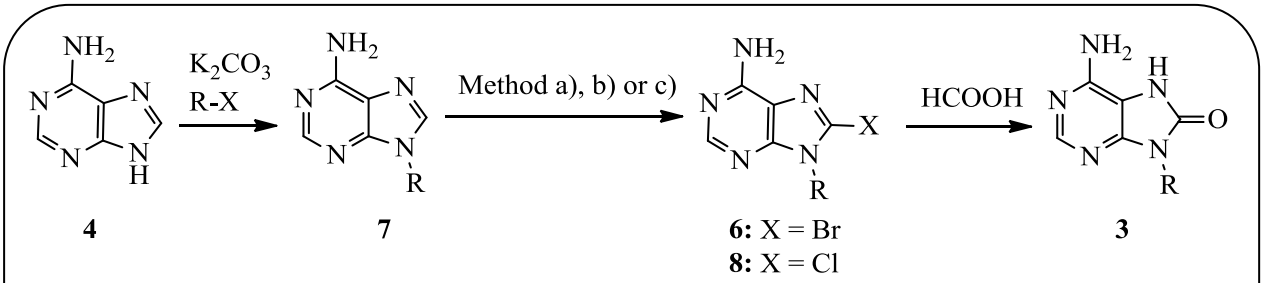

a) Br_2; b) NBS; c) LDA, then C_2Cl_6 or $BrCl_2CCCl_2Br$

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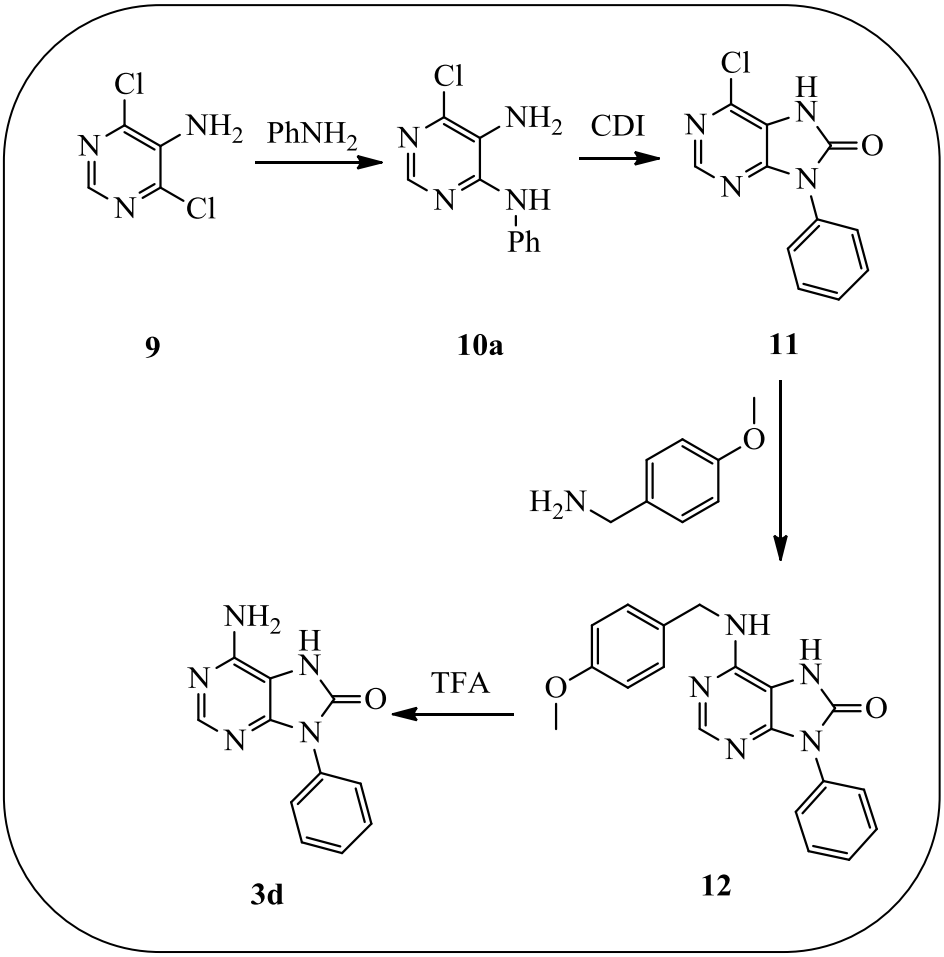
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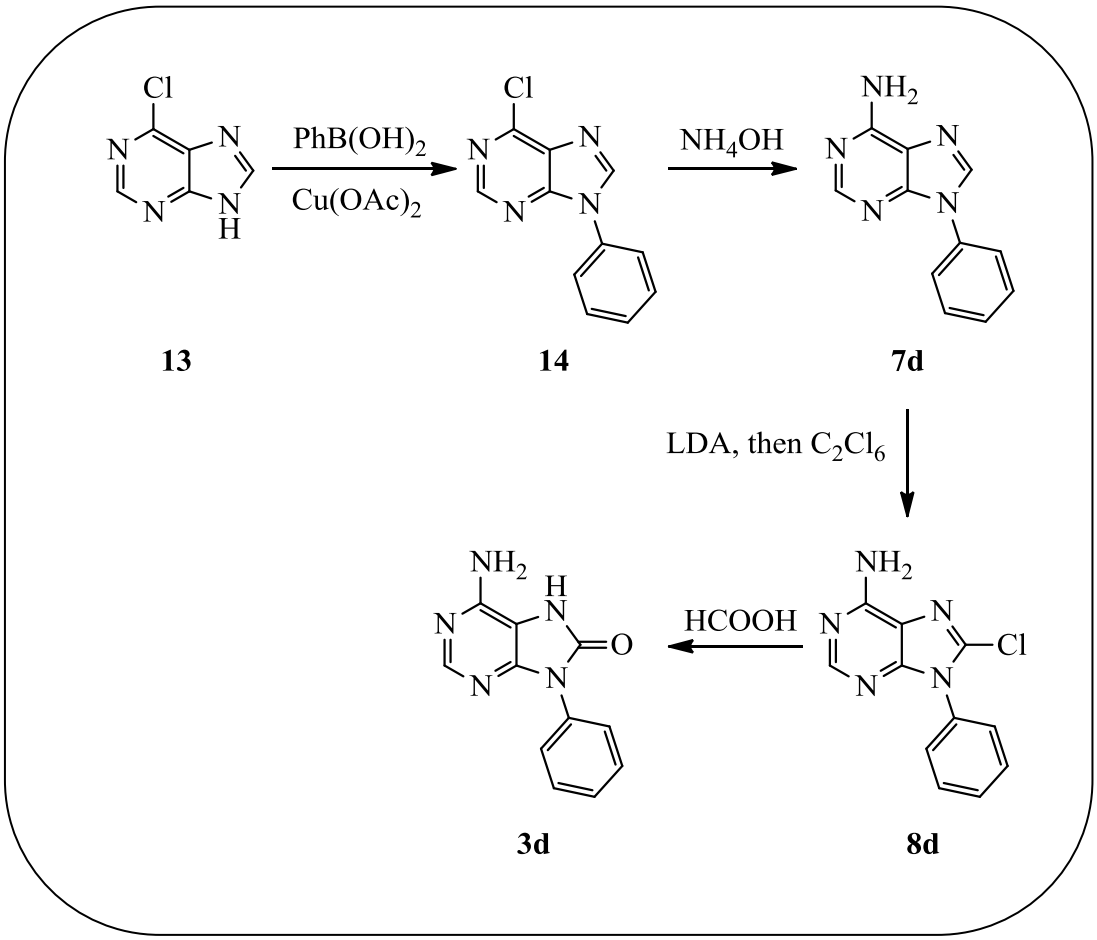
Strategy 5: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CHAPTER 1

1. BIOLOGICAL BACKGROUND

1.1. General

In Norway alone, there were 26,121 new cases of cancer registered at Kreftregisteret ("the Cancer Registry") in 2008, with prostate, female breast, colon and lung cancers being the most common types reported.[4] The World Health Organisation (WHO) reports that every year at least 7 million people die of cancer. Half of these deaths are avoidable, and mortality rates are higher in developing countries due to a lack of access to medical technology and treatment.[5] Although there already exists a wide range of treatments for different types of cancer, there is still room for improvement of survival rates, especially for certain types of cancer.[6]

Two types of established treatments directly cause damage to cancerous cells – radiation therapy (using ionizing radiation) and chemotherapy (treatment with drugs). A third common treatment is surgery to remove the cancerous cells. Both radiation therapy and chemotherapy cause damage to the DNA of cancer cells with the aim of causing cell dormancy or death in order to eliminate cancers completely or cause them to become benign. However, DNA repair mechanisms in cancer cells can inhibit the effectiveness of such methods by healing the damaged DNA in these cells.[6] There exists some research in this area, but there is still a need for further research targeting specific repair mechanisms to improve already established treatments – i.e. development of inhibitors of proteins that carry out DNA repair.[6]

The targeting of potential protein inhibitors is based on the principle that cancer cells will be affected by DNA damage more than normal cells due to the high rate of cell division and metabolism in the former. This could potentially result in higher rates of mutation, cell death and/or dormancy in the more active cancer cells. The current project has involved synthesis of target molecules that are intended to act as inhibitors for certain DNA glycosylases – human alkyladenine DNA glycosylase (hAAG) or human 8-hydroxyguanine DNA glycosylase (hOOG1); the former partakes in BER of alkylated adenines and the latter partakes in BER of 8-oxoguanine lesions.[7,8]

1.2. DNA Replication, Damage and Repair

1.2.1. DNA Replication

When cells reproduce, one important step of the cell cycle is DNA replication in preparation for cell division (mitosis). This process is complicated, involving many different proteins. One of these proteins causes the breaking of the hydrogen bonds between the bases in the DNA double helix at particular points known as "origins". Other proteins hold the unwound DNA strands in place creating a "bubble" in the DNA, resulting in the creation of two "replication forks". Each strand of DNA is then used as a template for the assembly of a new complementary strand by pairing the bases in the new strand with those in the template strand (Figure 3 and Figure 4).[9]

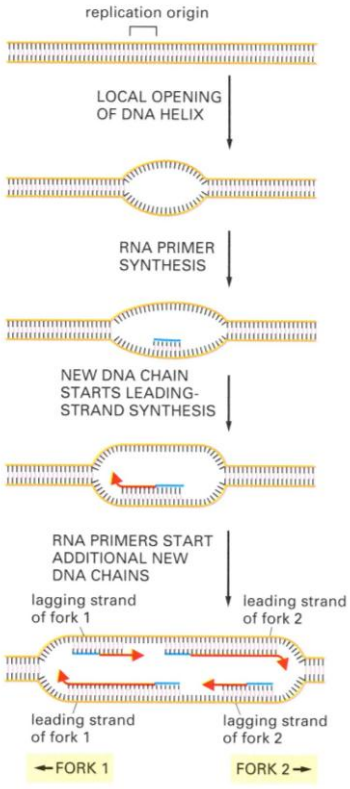


Figure 3. Diagram showing the major steps of initiating replication forks at replication origins and the use of the original DNA strands as templates for the new strands.[9]

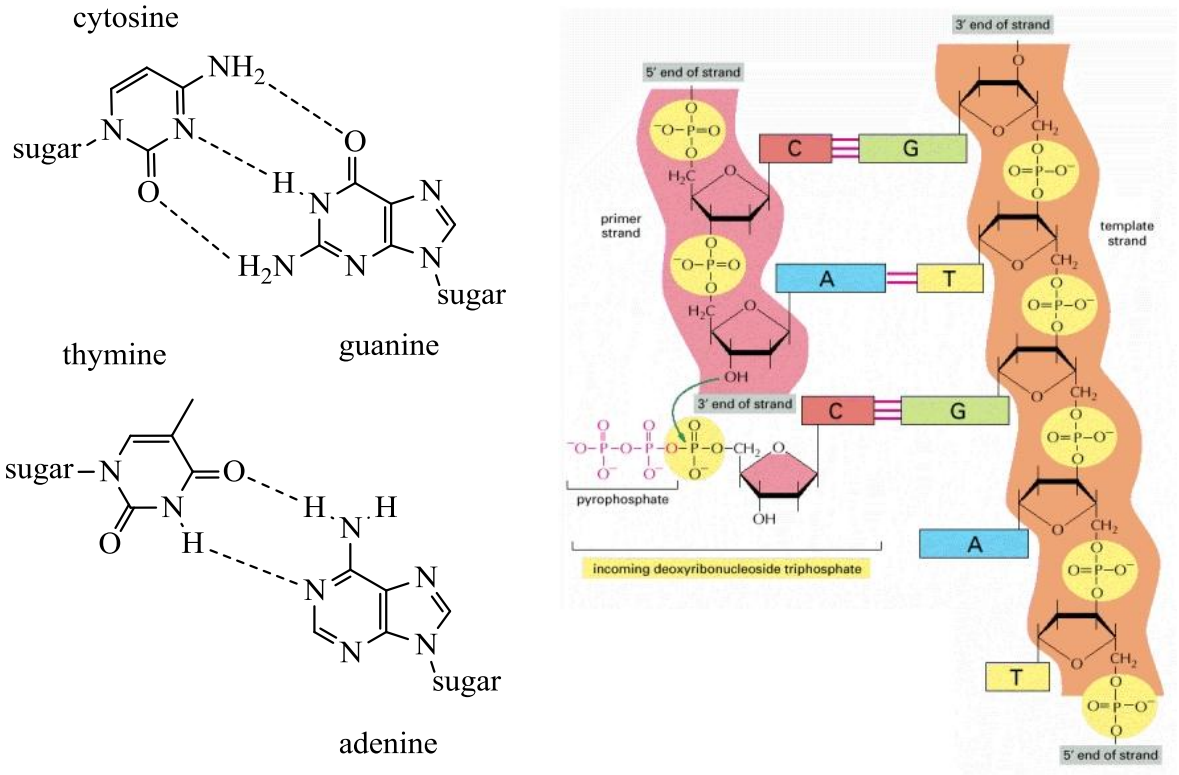


Figure 4. In DNA, nucleotides will only pair with one other nucleotide (i.e. form a "base-pair"). For example, adenine (A) will only pair with thymine (T), while guanine (G) will only pair with cytosine (C) because of the specific pattern of hydrogen-bonds between the nucleotides (left) – meaning that each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing (right).[9]

1.2.2. DNA Damage

There are many kinds of damage that can occur in human DNA that result in the improper function or death of cells, such as base modifications, single-strand breaks and double-strand breaks, replication lesions and DNA crosslinks.[10] These types of modifications to DNA, when uncorrected, can lead to events such as the deletion of a base pair or base-pair substitution in the strands during the replication process, which can cause problems in the cell cycle or mutations to the cell's DNA, resulting ultimately in cell death or dormancy.

Healthy cells and cancer cells have different properties, one of which is that cancer cells have a higher rate of cell division and metabolism.[10] Cancer treatments take advantage of this difference by causing DNA damage to all cells, but that affects cancer cells to a greater extent

to preferentially provoke cell death or dormancy in those cells.[9-11] In the current project, the targeted enzymes are known to carry out repair of alkylated adenine bases and oxidised guanine bases (base damage). They are also believed to repair other types of DNA damage.[12,13] In this section, we will have a look at the causes and repair of damaged bases.

In Figure 5 below are shown the bases found in DNA – guanine, cytosine, thymine and adenine – attached to their sugar moieties and phosphorous backbone. Shown with arrows are the sites where spontaneous damage can occur (see Figure 5).[9,14] In addition, DNA base modifications can be induced by oxidation, deamination, alkylation or the addition of bulky adducts to these bases through cancer treatments.[15] Examples of alkylating agents that are used in chemotherapy are shown in Figure 6.[10]

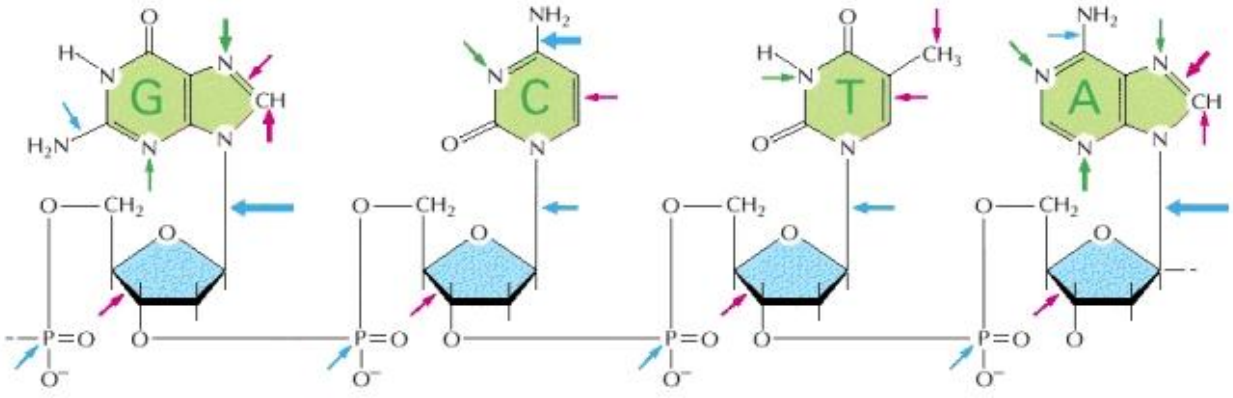


Figure 5. The sites on each nucleotide that are known to be modified by spontaneous oxidative damage (red arrows), hydrolytic attack (blue arrows), and uncontrolled methylation by the methyl group donor S-adenosylmethionine (green arrows) are shown, with the width of each arrow indicating the relative frequency of each event.[9,14]

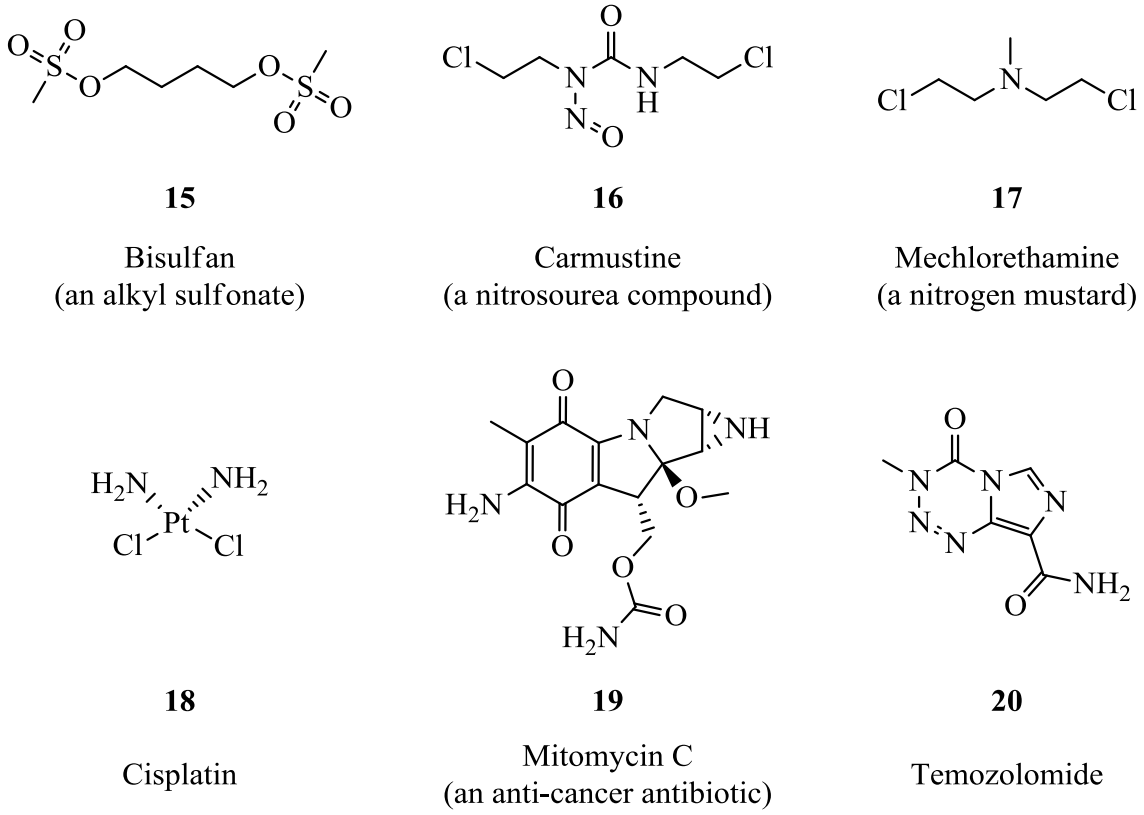


Figure 6. Examples of alkylating agents used in chemotherapy.[16]

One problem with radiation therapy and chemotherapy is that some of the damage inflicted on DNA is countered by DNA repair enzymes which repair damage caused by cancer therapy and reduce their effectiveness. In healthy cells, DNA repair mechanisms are essential for maintaining the structural integrity of DNA. However, in cancer cells, it is an unwanted hindrance to the effectiveness of the cancer treatment.

The hypothesis behind the current project is that inhibition of repair mechanisms will also take advantage of the differences between healthy cells and cancer cells and enhance the effect of cancer treatments on the latter. This idea has received some attention previously with respect to several repair proteins, but there remain many possibilities for new developments.[10] In Figure 7 below, proteins that have already been targeted for inhibition in order to enhance cancer treatment are shown in blue.

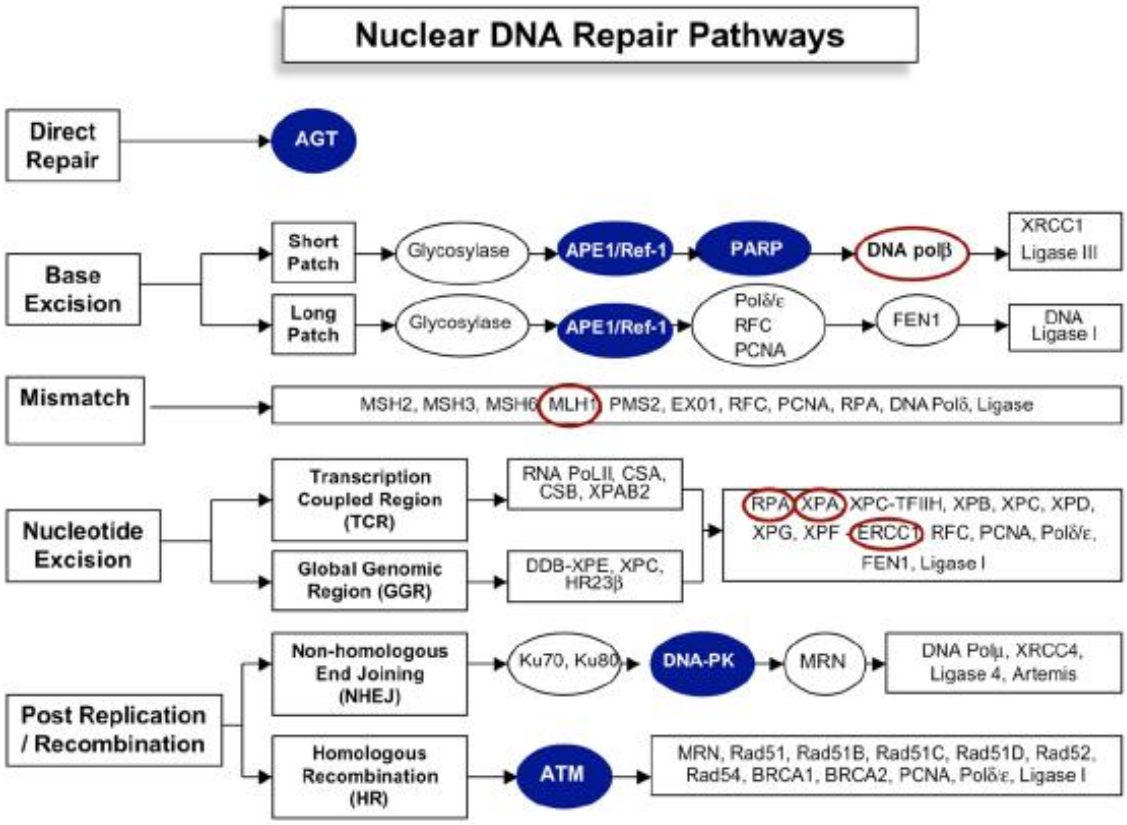
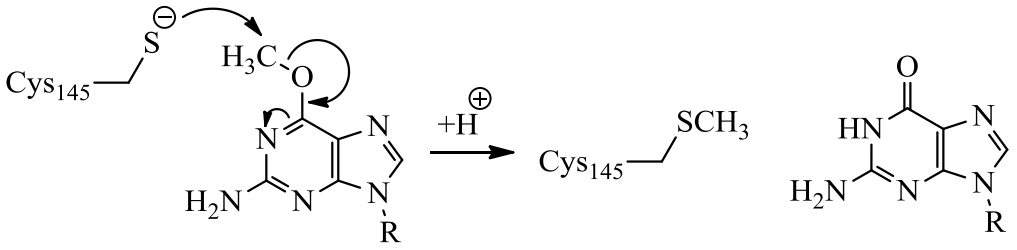


Figure 7. An overview of mammalian DNA repair pathways (not including all involved proteins). A blue background indicates proteins that are emerging as strong lead targets for cancer therapeutics and a red circle indicates that some data has been obtained.[6]

1.2.2.1. Direct Repair of Base Damage

Direct repair or direct reversal of DNA damage is a relatively easy mechanism and is undertaken by the AlkB enzymes and O^6-alkylguanine DNA methyltransferase (AGT) protein.[17] AGT transfers an alkyl group from an alkylated lesion on a DNA base to an active cysteine site. The AGT protein is consumed in the mechanism and thus would be more amenable to inhibition than a catalytic enzyme (Scheme 1).[18]



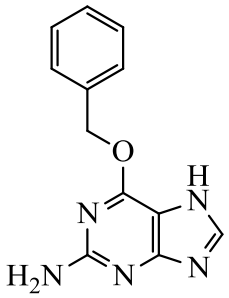
21a **21**

R = sugar link to phosphate backbone

Scheme 1. Mechanism of the direct repair of an alkylated guanine (**21a**) by AGT.[18]

The AlkB enzymes carry out the same procedure through an alternative oxidative dealkylation process. Inhibition of these mechanisms are favourable in combination with alkylating agents used in chemotherapies such as alkyl sulfonates, nitrosourea compounds, temozolomide, nitrogen mustard, Mitomycin C and Cisplatin (see Figure 6 above).[10]

AGT was one of the first DNA repair targets identified for inhibition.[6] O^6-Benzylguanine (BG) (Figure 8) has been shown to be a potent inhibitor of AGT and increases the potency of chloroethylating and methylating agents. BG binds to AGT and transfers a benzyl group to the active cysteine site of the protein, effectively "killing" the protein.[19]



21b

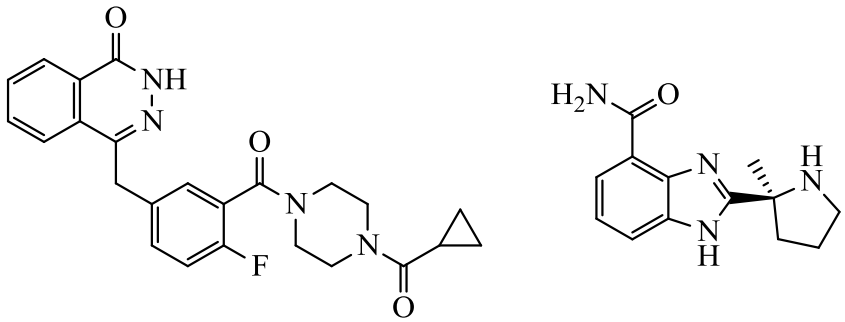
Figure 8. Structure of AGT inhibitor, O^6-benzylguanine (**21b**).

1.2.2.2. Base Excision Repair of Base Damage

When damage to a base occurs, the most common mechanism for repair is through BER, which repairs DNA bases that have been damaged through oxidation, alkylation, hydrolysis, or deamination. A family of enzymes known as DNA glycosylases are responsible for identifying and excising specific lesion bases, such as 8-oxoadenine, from the strand,[8] and the enzymes targeted in the current project belong to this family.

After excision, a non-basic site is left at the point of excision and PARP proteins indicate to other proteins where a DNA strand break exists so that these proteins can carry out the actual reparation.[6] The enzyme Apurinic endonuclease 1/Redox factor-1 (APE1/Ref-1) and subsequently enzymes β-polymerase and DNA Ligase III/XRCC1 then restore the site by reinstating the base moiety.[6] There are thus several steps at which BER can be inhibited.

Inhibition of the action of PARP proteins can make cancer treatments more effective, as a failure to recognise strand breaks and signal the repair proteins to act results in a dysfunctional BER mechanism.[20] Several PARP inhibitors have been developed based on the biologically-produced nicotinamide and the compound 3-aminobenzamide. These inhibitors are usually monoaryl amides or cyclic amides with one or more rings (see e.g. Figure 9). They act competitively by blocking access to the catalytic part of the enzyme or blocking the attachment of the enzyme to DNA.[21]



22 **23**

Figure 9. Examples of PARP inhibitors – KU-0059436 (**22**) [22] and ABT-888 (**23**) [23].

Several compounds have been reported as inhibitors for APE1/Ref1 and these improve the toxicity of the chemotherapy drug temozolomide (Figure 6, Compound 11) in certain types of

cancer. One of these compounds, methoxyamine, binds to the sugar in the abasic site left from the excision preventing the action of APE1/Ref1 and subsequent proteins.[6]

1.2.3.1.3. Nucleotide Excision Repair and Mismatch Repair of Base Damage

NER and MMR are ways in which damage that affects the overall structure of DNA (e.g. cross-link damage) can be repaired.[6] Nucleotide excision repair (NER) is important for the repair of large bulky lesions that distort the helix structure. The dual incision mechanism requires a large number of proteins that recognise, excise and replace a large part of the strand containing the lesion. The bulky lesion is first recognised by a group of proteins and a small opening may be created at this stage. The structure is then opened further by another protein and two structure-specific endonucleases are involved in the dual incision which removes the damage in a long strand. Repair synthesis is then carried out to repair the patch by a DNA ligase.[24]

Mismatching of nucleotides, such as base substitution mismatches and insertion-deletion mismatches, can arise during DNA synthesis and requires repair in order to avoid fixed mutations – where the mismatched base becomes established in the DNA sequence. Mismatch repair (MMR) is similar to NER in that several proteins work together to identify the mismatch, excise a long single strand around the mismatch and to patch up the remaining DNA, but different proteins are involved.

One inhibitor of NER is Fludarabine, an adenine-derivative that has been shown to be effective in the treatment of chronic lymphocytic leukaemia (Figure 10). This compound has proved to provoke a higher response rates to cancer therapy use of alkylating agents alone.[25]

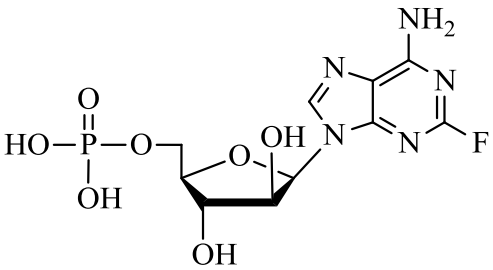


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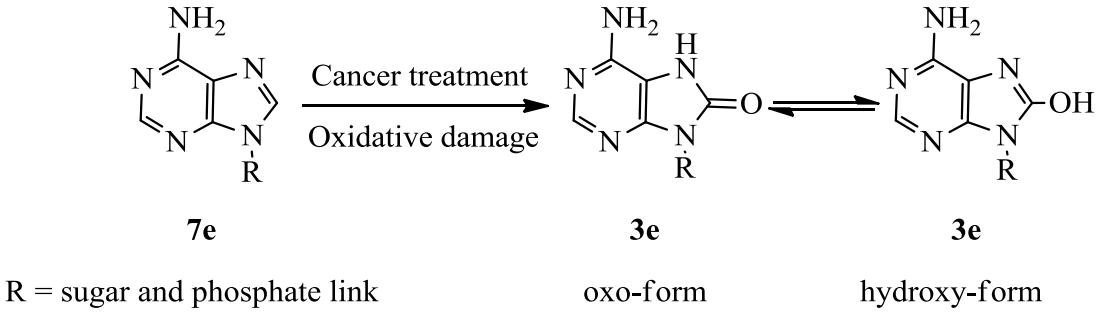
Figure 10. Structure of NER inhibitor, Fludarabine (**24**).

1.3. Choice of Target Molecules

Although research on some of the many proteins involved in repairing DNA damage is being carried out, there is still much room for investigating and developing substances that will inhibit the action of repair proteins and sensitize cells to the effects of anti-cancer treatment.

The design of the target molecules has been carried out in collaboration with Research Fellow Bjørn Dalhus at the Institute for Clinical Biochemistry at the Rikshospitalet-Radiumhospitalet Medical Centre. The initial *in vitro* biological testing is taking place at that institute, and the results that have been obtained so far are included in the thesis and further testing is planned for our compounds.

The main aim of the current project was to synthesise molecules that have the potential to inhibit BER at the first stage – i.e. interfere with the action of DNA repair glycosylases that recognise and excise 8-oxoadenine lesions (**3e**) (Scheme 2). Target molecules were therefore designed to mimic the form of this oxidative lesion.



Scheme 2. Oxidative lesion created by oxidation of the adenine moiety (**7e**) in DNA.

The target molecules in this project have the aim of inhibiting the action of repair enzyme by being similar in the structure of 8-oxoadenine lesions (**2**) such that they will potentially interfere with the action of glycosylase enzymes. Target molecules are therefore 8-oxoadenine derivatives with different substituents attached to *N*-9.

The following molecules were targets for this project (see Figure 11):

- 6-amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**)
- 6-amino-9-cyclopentyl-*7H*-purin-8(*9H*)-one (**3b**)
- 6-amino-9-(cyclohexyl)methyl-*7H*-purin-8(*9H*)-one (**3c**)
- 6-amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**)

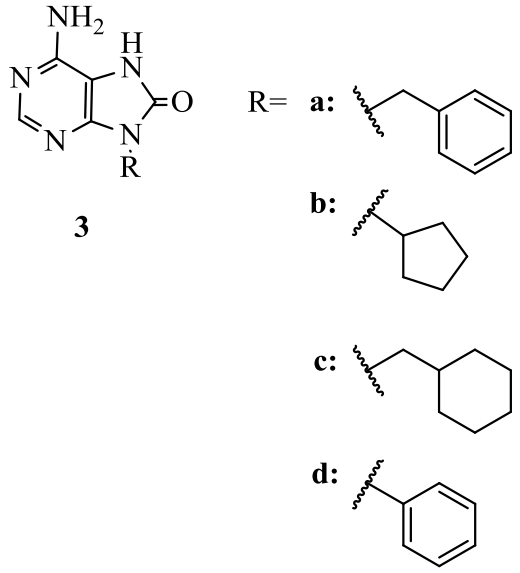


Figure 11. The target molecules for the current project.

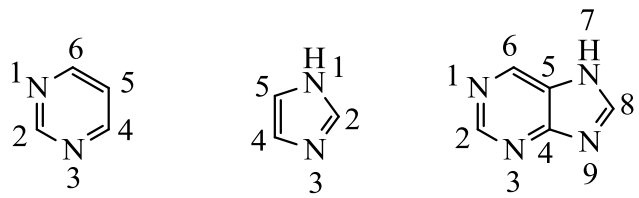
CHAPTER 2

2. INTRODUCTION TO THE CHEMISTRY

2.1. General

The methods encountered in the course of the work done during the current project were *N*-alkylation and *N*-arylation, nucleophilic aromatic substitution, lithiation, bromination, ring-closing and hydrolysis. As will be shown in the thesis, the order in which these transformations are carried out has ramifications for yields, regioselectivity and can affect the difficulty of purification.

In the current thesis, the standard International Union of Pure and Applied Chemistry (IUPAC) numbering system that is used for pyrimidine, imidazole and purine will be employed (see Figure 12).[26,27] IUPAC names will also be given for each compound, but where appropriate a simplified version may be used to allow for ease of reading.



25 **26** **27**

pyrimidine imidazole purine

Figure 12. Customary numbering system for pyrimidines, imidazoles and purines.

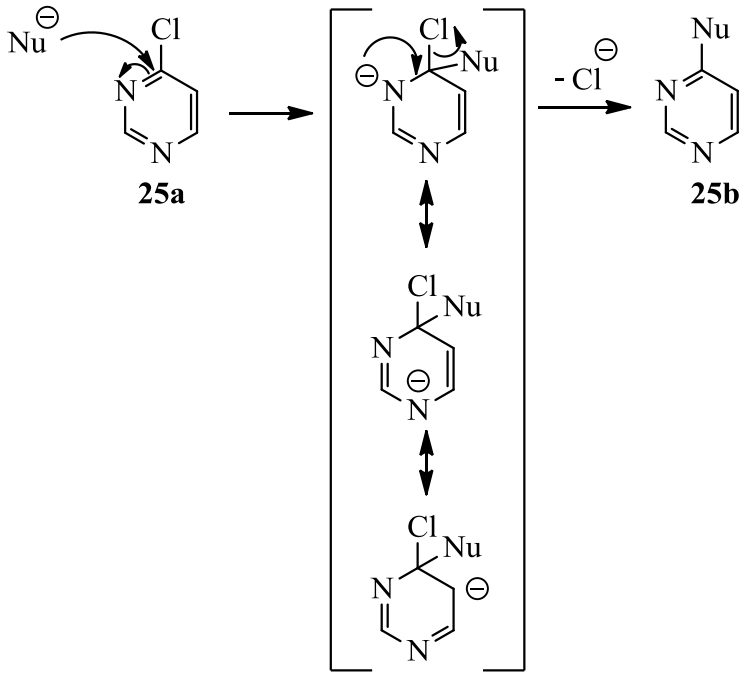
Purines and pyrimidines are amenable to a wide variety of chemistry including reactions with nucleophiles, electrophiles and alkylating agents, in addition to, coupling reactions and halogenation.

2.2. Nucleophilic and Electrophilic Substitutions with Purine, Pyrimidine and Imidazole

Diazines, including pyrimidine and imidazole, contain two nitrogen atoms, hence the name. These types of ring systems are more electron-poor than, for example, pyridine or benzene rings, due to the presence of electron-withdrawing nitrogen atoms in the former. As a result, nucleophilic attack on carbon atoms in the ring is easily carried out and electrophilic attacks are much more difficult than for pyridine or benzene.[28]

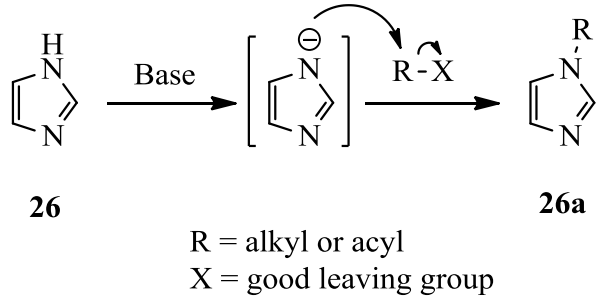
The presence of a halogen on the position α to a ring-nitrogen of any hetroaromatic compound will increase the ease of nucleophilic attack at that position by decreasing the electron-density on the carbon to which it is attached, compared to having a hydrogen atom in the same position. These types of reactions involving 2-, 6- and 8-halopurines and 4- and 6-halopyrimidines with nucleophiles are of vital importance to the synthesis of functionalised purines.[28-30] For example, a fluoro, chloro or bromo group will activate its neighbouring carbon for S_NAr attack while a bromo or iodo group can be useful in coupling reactions because of their weaker carbon-halogen bond, which allows for easier insertion of the catalytic metal species.[28]

Nucleophilic attack on halogenated aromatic compounds, where a nucleophile is substituted with a halogen, does not undergo an S_N1-type reaction due to the dissociation of the aryl halide being energetically unfavourable. Neither does it undergo an S_N2-type reaction as it is sterically shielded from backside attack. This type of reaction, nucleophilic aromatic substitution (S_NAr), occurs rather through a addition/elimination mechanism where the nucleophile attacks the aromatic ring to give a stabilised carbanion intermediate which then regains aromaticity through the elimination of chloride (Scheme 3).[31]



Scheme 3. Mechanism of S_NAr on 4-chloropyrimidine (**25a**) to a 4-substituted product (**25b**).

Imidazole is a more electron-rich ring system than pyrimidine because it is a six-electron aromatic system spread over only five atoms.[28] This ring is thus able to react with electrophiles, as well as still being reasonably open to nucleophilic attack. The former includes reactions with alkylating and acylating agents on nitrogen and halogenation on carbon (Scheme 4). The latter is possible because the intermediate of nucleophilic attack on any halogenated carbon (preferably C-2) can also be resonance-stabilised onto the neighbouring nitrogen atoms (*N*-1 and *N*-3), similar to the case of pyrimidine (Scheme 3).[28]



Scheme 4. Mechanism of a reaction of imidazole (**26**) with an electrophile (e.g. alkyl or acyl halide).

Purines consist of a fused-ring system that displays a combination of the characteristics of pyrimidine and those of imidazole. Nucleophilic aromatic substitutions are possible on carbons in both rings, although the reactivity of each position is dependent on substituent-effects. However, reactions of purines with electrophilic reagents (e.g. halogenation reactions) on carbon only occur in the imidazole ring (in other words, on C-8).[28]

2.3. *N*-Alkylation of Purines

One of the challenges with *N*-alkylation of purines is that purines can exist in several tautomeric forms in both neutral and basic conditions (see Figure 13), resulting in the possibility of several regioisomeric products being produced in any single reaction. According to literature,[28] the *7H* and *9H* tautomers are present in equal amounts in solution and the other two tautomeric forms are not present in significant amounts, but the distribution of the tautomers is dependent on both solvent effects and temperature and can also vary depending on the alkylating agent.[32,33]

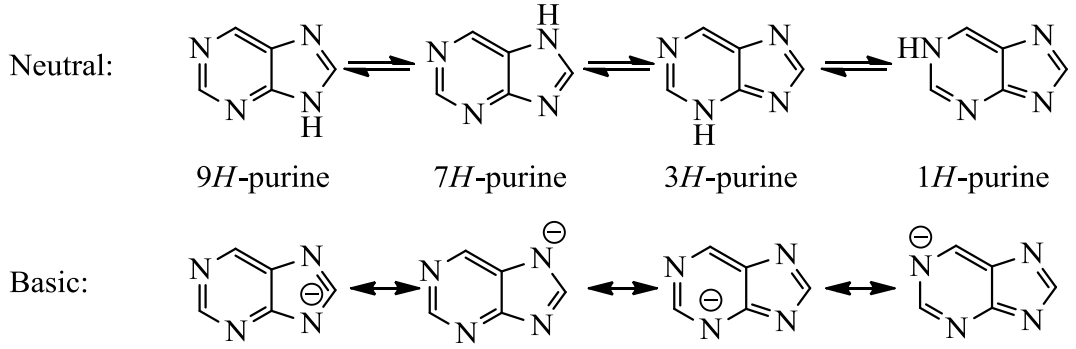
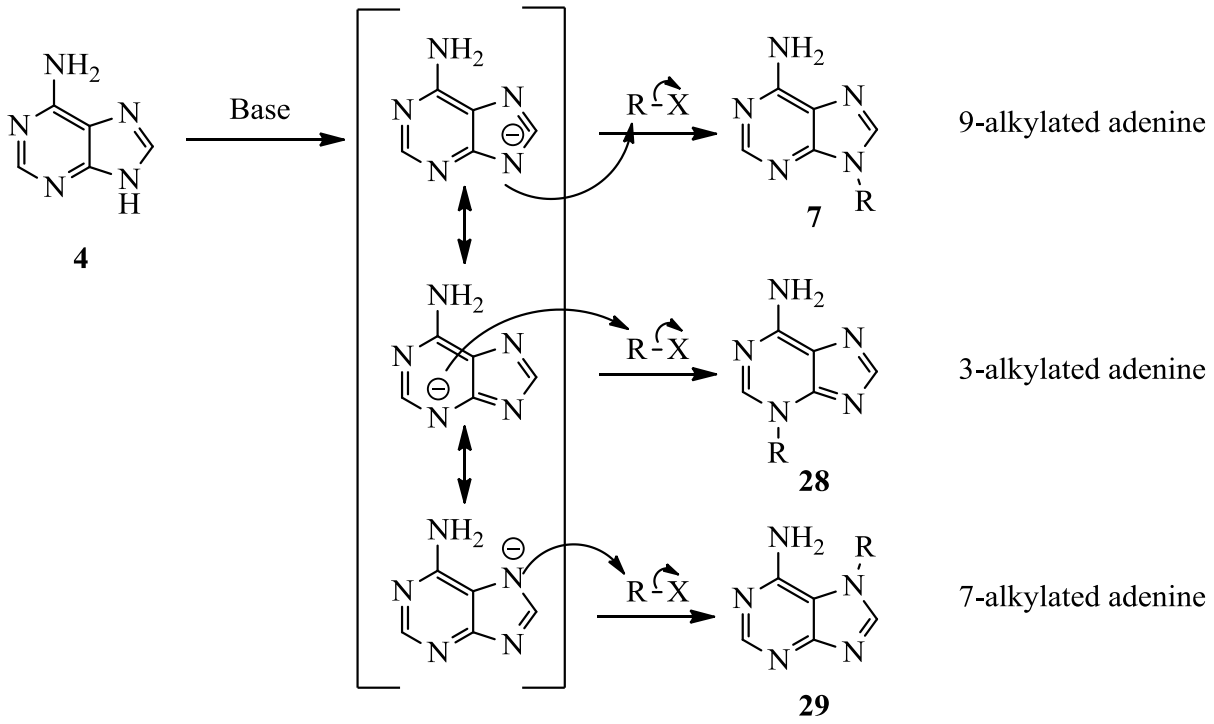


Figure 13. Tautomeric forms of purine (**27**) under neutral conditions and resonance forms under basic conditions.

The conditions of the alkylation can play a large part in determining the result – including the type of base used, temperature and type of alkylating agent. For example, reaction of adenine with an alkyl halide in neutral conditions gives 9-alkylated (**7**) and 3-alkylated (**28**) adenine while in the presence of a base is reported to give mainly substitution on *N*-9 with the 7-alkylated isomer (**29**) being an additional (minor) product in most cases.[28,34-36] The

1-regioisomer has not been reported to be formed. It has been suggested that ratio of the regioisomers is dependent on the transition state of the particular reaction.[33]

The general mechanism for deprotonation with a base, followed by nucleophilic attack on an alkyl halide is shown below (Scheme 5).



Scheme 5. Scheme for deprotonation and attack of the anion on an electrophile.

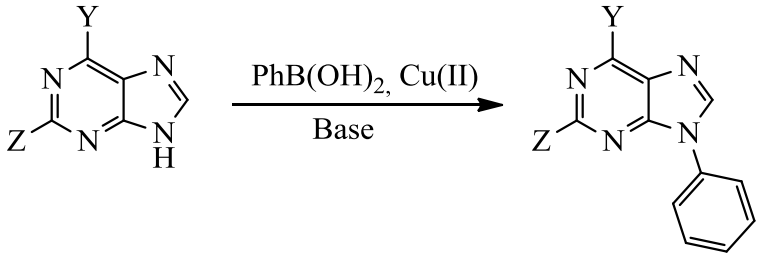
Alkylation of 8-bromoadenine in the presence of a base has been reported to give only 9-subsituted products.[34,37]

2.4. *N*-Arylation of Purines

N-Arylation of adenine requires the creation of a carbon-nitrogen bond between two aromatic rings. Copper-mediated cross-coupling reactions between heteroatoms (C-N or C-O) using boronic acids have been discovered to be a mild, efficient and versatile method (Chan-Lam-Evans coupling) and have the advantage of tolerating both air and room temperatures.[38-40]

There are literally hundreds of boronic acids commercially available so this type of reaction has many potential uses in the functionalization of purines.[40] To the best of our knowledge, palladium-catalysed or nickel-catalysed coupling of an aryl boronic acid or aryl halide to a ring-nitrogen on a purine has not been reported, however, C-C coupling has been observed.[41]

Arylation has been reported to occur regioselectively on the *N*-9 position of adenine, 6-chloropurine and 2,6-dichloropurine using phenyl boronic acid in the presence of copper(II) acetate and a base (Scheme 6).[42-44].

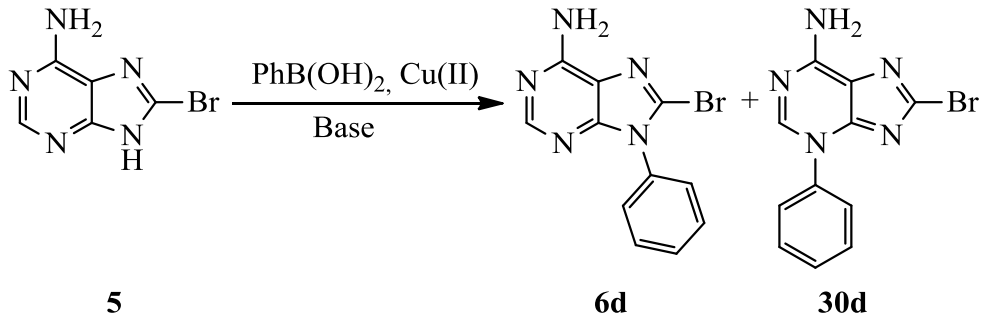


4: Y = NH_2, Z = H
13: Y = Cl, Z = H
13a: Y = Cl, Z = Cl

7d: Y = NH_2, Z = H
14: Y = Cl, Z = H
14a: Y = Cl, Z = Cl

Scheme 6. Chan-Lam-Evans coupling reactions on adenine (**4**), 6-chloropurine (**13**) and 2,6-dichloropurine (**13a**) using copper(II) acetate and phenyl boronic acid to give the arylated compounds.

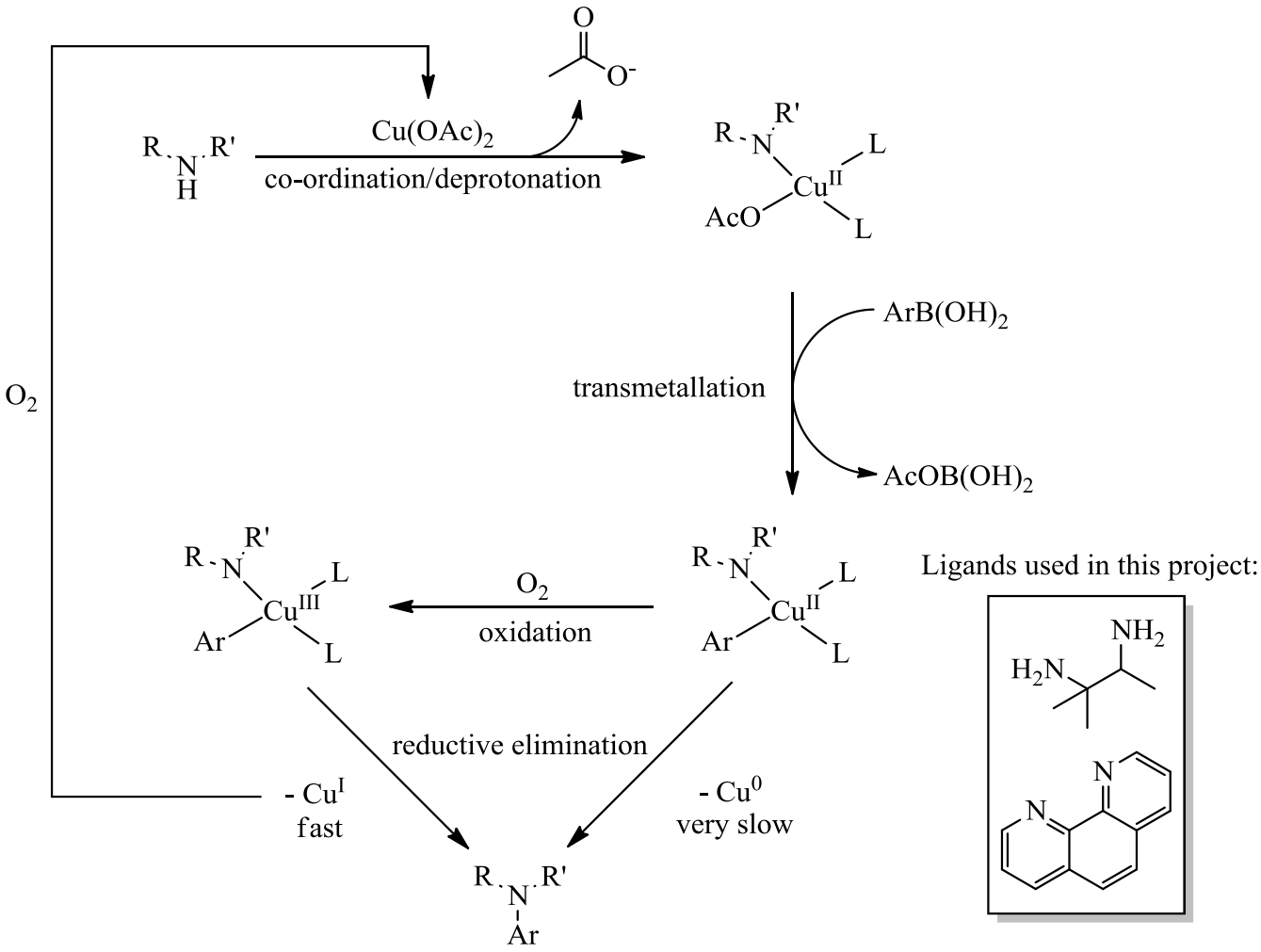
In the case of 8-bromoadenine (**5**), arylation has been reported to be less selective with both 9- and 3-arylated compounds (**6d** and **30d**) being produced, and the latter regioisomer as the major product.[42,45] This regioselectivity may be due to either electronic or steric effects of the bromine substituent on C-8.



5 **6d** **30d**

Scheme 7. Chan-Lam-Evans coupling reactions on 8-bromoadenine (**5**).[42]

The mechanism for this copper-mediated coupling reaction (C-N bond forming) is not as well-established as that for palladium-coupling reactions (C-C bond forming). Scheme 8 below depicts the routes through which copper-mediated coupling reactions with stoichiometric amounts of copper is thought to occur between a secondary amine and an aryl boronic acid.[40,46] This mechanism may be, however, relevant for all *N*-arylations of heterocyclic compounds using boronic acid.



Scheme 8. Mechanism describing Chan-Lam-Evans copper-mediated cross-coupling between an amine and aryl boronic acids and possible ligands.[40,46]

The mechanism begins with deprotonation of the amine and co-ordination to the copper complex by exchange of one acetate unit with the substrate. This results in rapid dissolution of the copper(II) acetate, which is insoluble in dichloromethane – the preferred solvent in these reactions following a solvent study of *N*-arylation of morpholine.[40] Transmetallation of the arylboronic acid with the complex is the next step resulting in the purine-aryl-copper(II) complex which can undergo reductive elimination to the coupled product and copper(0). However, this step is very slow as the heterocycle binds tightly to copper. Thus, there is a high probability that the copper(II) complex is first oxidised by elemental oxygen in the air to a copper(III) complex with undergoes a much faster reduction/elimination reaction to give copper(I) and the desired product.[40] The copper(I) can then be easily oxidised to regenerate copper(II) and complete the cycle. The reaction is thus facilitated by oxygen at two stages and it is not only convenient that this reaction tolerates air but benefits from an open reaction vessel.[40]

2.5. Halogenation of Purines

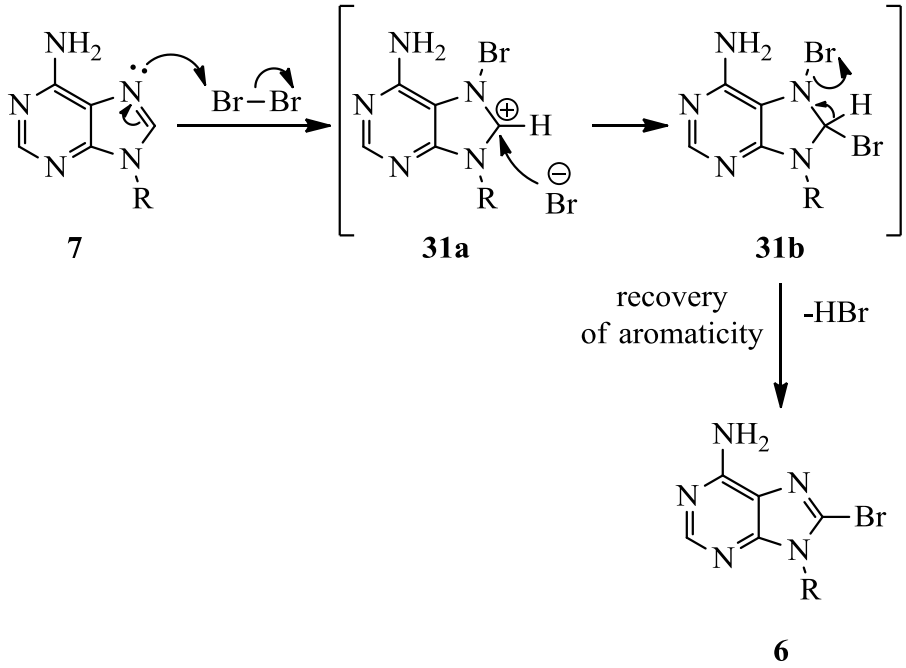
Three methods of bromination of purine substrates were encountered in the course of the current project. These were:

- Bromination with liquid bromine (both in the presence and absence of water)
- Bromination with *N*-bromosuccinimide
- Halogenation *via* lithiation with lithium diisopropylamide and subsequent capture with a source of electrophilic halogen

2.5.1. Bromination with Liquid Bromine

Bromination with liquid bromine is suggested to involve addition of bromine across the imidazole double-bond followed by elimination of hydrogen bromide (Scheme 9).[28] Purine itself does not undergo bromination on carbon, but adenosine derivatives undergo fluorination, chlorination and bromination at C-8. The proposed mechanism, shown with bromine in this example, follows the usual steps of electrophilic aromatic substitution (Scheme 9).[28]

The mechanism involves electrophilic attack by, most probably, the lone electron pair on *N*-7 on bromine creating the *N*-bromopurinium salt (**31a**). This salt can undergo nucleophilic addition of the free bromide anion, followed by elimination of hydrogen bromide to give the brominated product (**6**).[28] It is possible that the driving force in this last step could be the regaining of aromaticity in the imidazole ring.



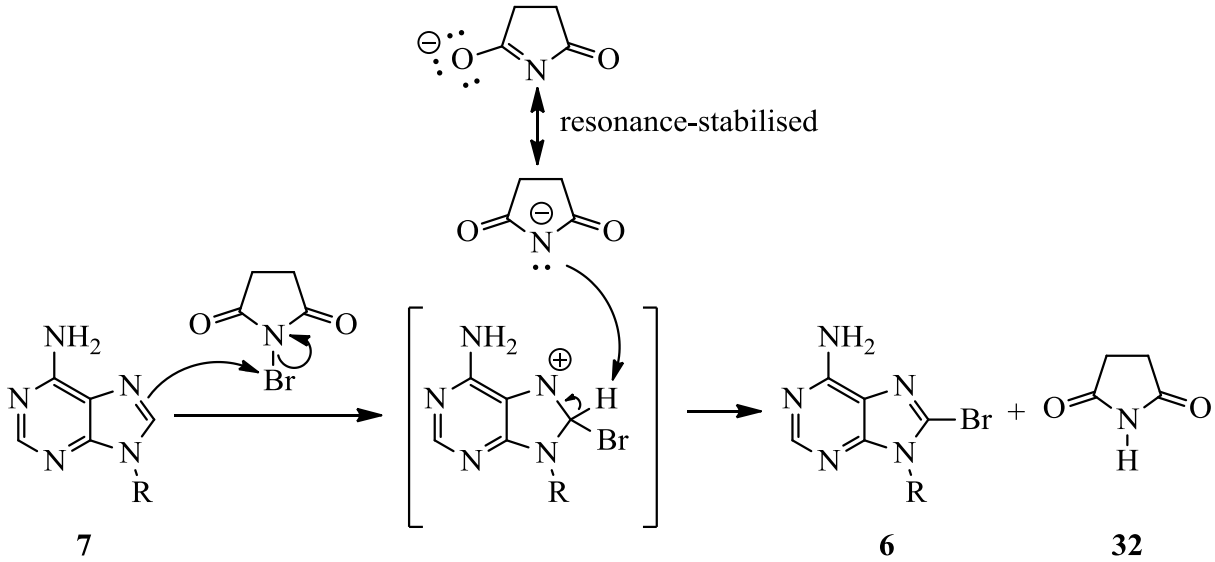
Scheme 9. Mechanism of bromination of 9-substituted adenines (**7**) through electrophilic attack on bromine and elimination of hydrogen bromide to give the 8-brominated product (**6**).

2.5.2. Bromination with *N*-Bromosuccinimide

N-Bromosuccinimide (NBS) is a brominating agent that can promote reactions *via* two different reaction mechanisms – through radical reactions and also electrophilic addition (Scheme 10).[47,48] NBS is known to brominate allylic positions through the radical route in the presence of a radical initiator (Wohl-Ziegler reaction). In the absence of such an initiator and/or where an electron-rich substrate is used, NBS is a convenient source of what can be considered to be cationic bromine for electrophilic substitution reactions.[48]

The mechanism below, bromination of 9-substituted purines with NBS, is an example of the latter route. NBS provides an electrophilic source of bromine to the reaction because the

strongly electron-withdrawing succinimide group pulls electron-density from the bromine atom, creating a partial positive charge on the halogen atom. This leaves the bromine vulnerable to attack by the nucleophilic purine, which results in an electrophilic addition of the purine. Removal of a proton by the succinimide anion gives the re-aromatised product.[47] The reaction is regioselective for the C-8 position because the imidazole-ring is electron-rich while the pyrimidine ring is electron-poor.



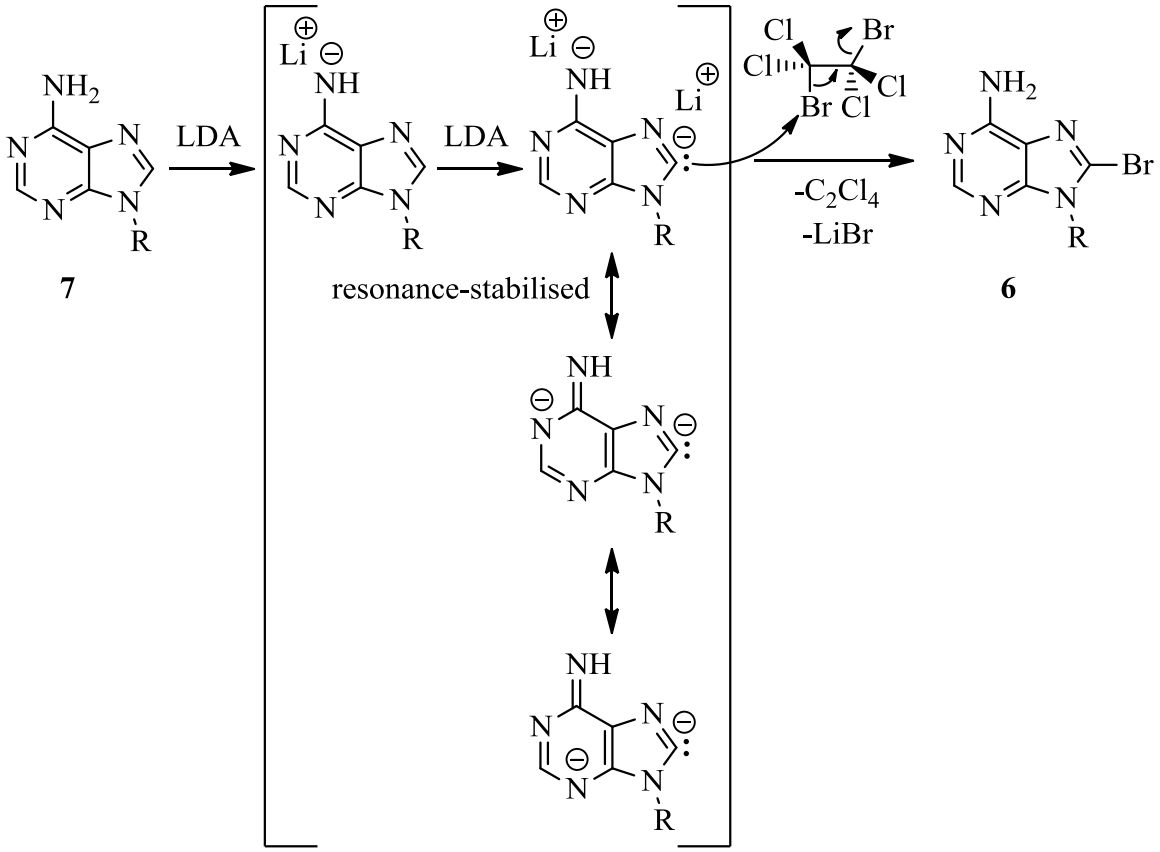
Scheme 10. Mechanism of bromination of compounds **8** with NBS to give compounds **6** and succinimide (**32**).

2.5.3. Lithiation and Capture with an Electrophilic Source of Bromine

Halogenation using LDA involves deprotonation at C-8 and the formation of a lithiated species, followed by capture by an appropriate electrophile. This method has been employed extensively in the Gundersen group at UiO, and has been shown to be efficient on 9-benzyl-6-chloropurines, with certain substituents on the benzyl ring, and has had some success on a limited range of 9-substituted adenines.[49]

Scheme 11 shows the deprotonation of 9-substituted adenines (**7**) and creation of a lithium salt using lithium diisopropylamide (LDA). As adenines have an amino group which has a relatively acidic proton, this proton is deprotonated first followed by the deprotonation of the C-8 hydrogen, resulting in a dilithiated adenine salt.[50] The presence of this amino group

therefore means that more of the base is required to deprotonate at C-8, than if it had not been present.



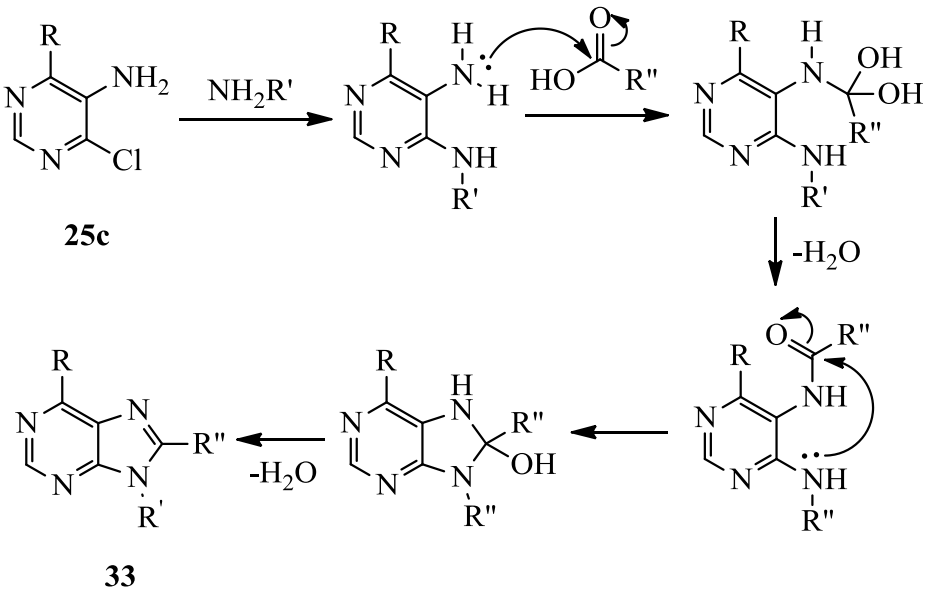
Scheme 11. Mechanism for deprotonation of compounds **7** and subsequent capture with 1,2-dibromo-1,1,2,2-tetrachloroethane to give compounds **6**.

The negative charge on the N^6 can be delocalised into the pyrimidine ring giving conjugated tautomers. This is not the case for the negative charge on the C-8 carbon, which is unable to be stabilised by resonance. In addition, the five-membered imidazole ring is more electron-rich than that six-membered ring. These factors result in the increased nucleophilicity and reactivity of C-8 compared with the deprotonated amino group (N^6). This difference in reactivity is thought to be the basis of the regioselectivity of the method. Treatment of the lithiated species with, for example, 1,2-dibromo-1,1,2,2-tetrachloroethane molecule results in the 8-brominated product.[51]

2.6. Preparation of Purines by Ring-Closing of Pyrimidines and Imidazoles

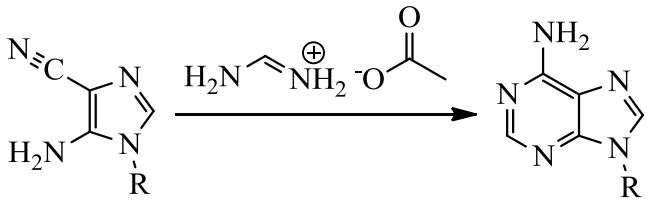
Sometimes it is desirable to functionalise either a pyrimidine or imidazole compound first then ring-close the other ring to construct the purine ring system. This can be necessary when it is difficult to obtain good yields or regioselectivity from functionalization of an intact purine ring system.

For example, 4-chloro-5-aminopyrimidines (**25c**) can be aminated with a primary amine *via* an S_NAr-type reaction to give substituted 4,5-diaminopyrimidines which can then be reacted with carboxylic acids or similar derivatives to give 8-substituted purines (**33**) (Scheme 12).[28,52]



Scheme 12. Mechanism of amination and ring-closing to obtain purine ring system (**33**) using carboxylic acid derivatives. R''=H, alkyl, etc.

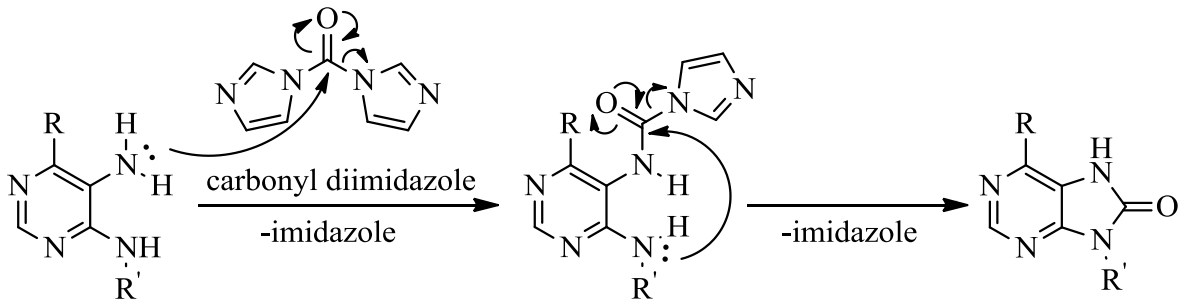
Alternatively, 4-amino-5-cyano-1-substituted-imidazoles may be treated with formamidine acetate to give 9-substituted-adenines (Scheme 13).[53]



26b **7**

Scheme 13. Reaction of 4-amino-5-cyanoimidazole (**26b**) with formamidine acetate to give 9-substituted adenines (**7**).

It should also be mentioned that an oxo- functional group can be obtained by ring-closing 4,5-diaminopyrimidines with carbonyldiimidazole (Scheme 14).

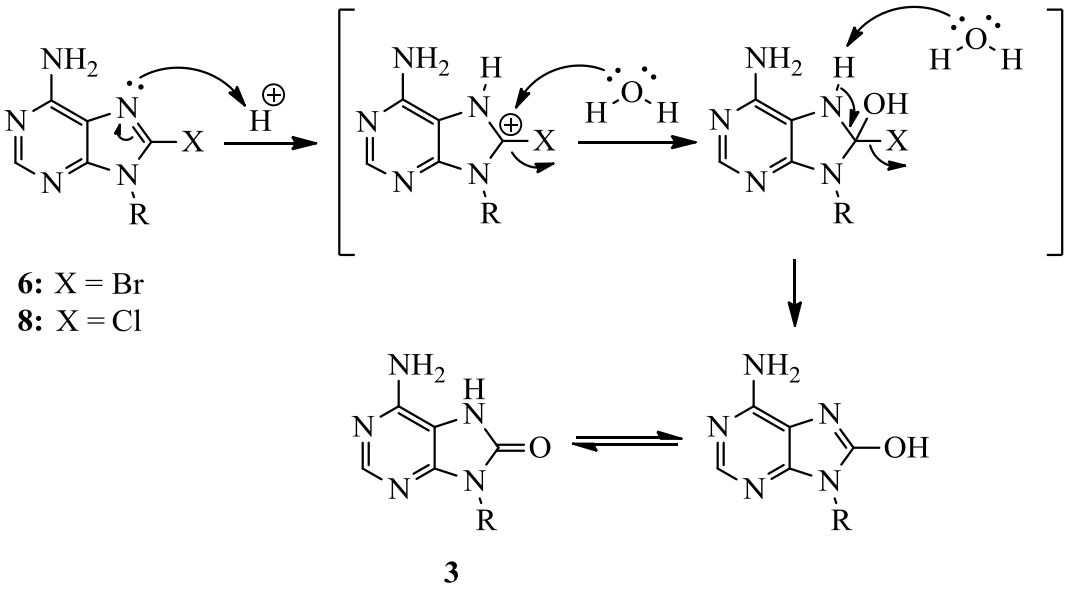


25e **34**

Scheme 14. Mechanism for the reaction of 4,5-diamino-pyrimidines (**25e**) with carbonyldiimidazole to give 8-oxopurine compounds (**34**).

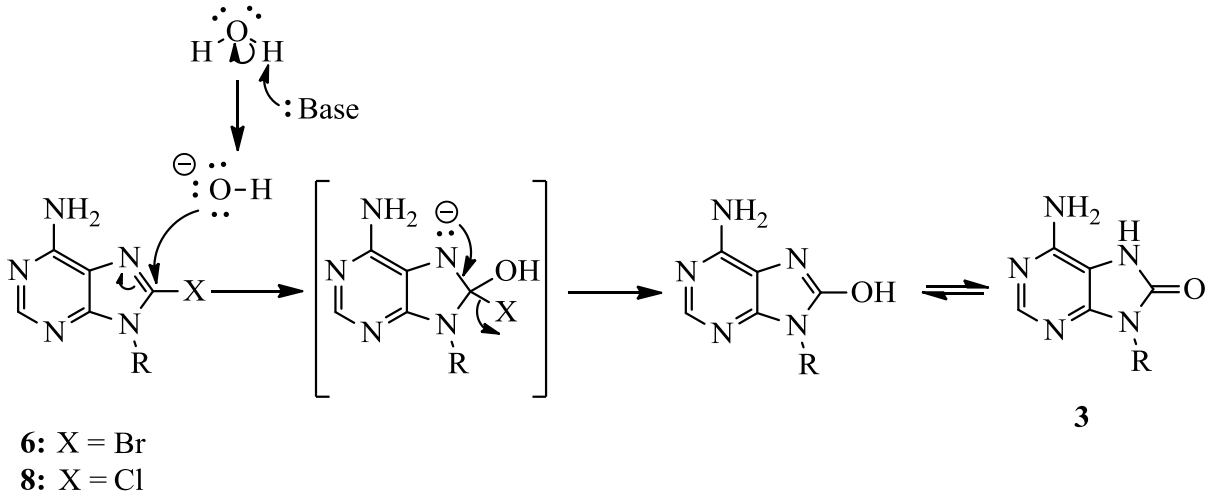
2.7. Hydrolysis of Halogenated Purines

8-Halopurines may be converted to 8-oxopurines by acid- or base-catalysed hydrolysis.[54] In the case of acid-catalysed hydrolysis (Scheme 15), a proton adds to the *N*-7 of the purine and the double-bond electrons transfer to the nitrogen, creating a positive charge on C-8. A water molecule can then attack the nucleophilic C-8 to create a tetrahedral intermediate, which can then eliminate a water molecule to obtain the 8-oxopurine derivatives (**3**).



Scheme 15. Assumed mechanism of acid-catalysed hydrolysis of 9-substituted-8-haloadenines (**6** or **8**) to give 9-substituted-8-oxoadenines (**3**).

In the base-catalysed reaction (Scheme 16), the base extracts a proton from a water molecule, giving an activated hydroxy group, which can then attack the C-8 of the halopurines and result in an S_NAr reaction to give compounds (**3**).[28]



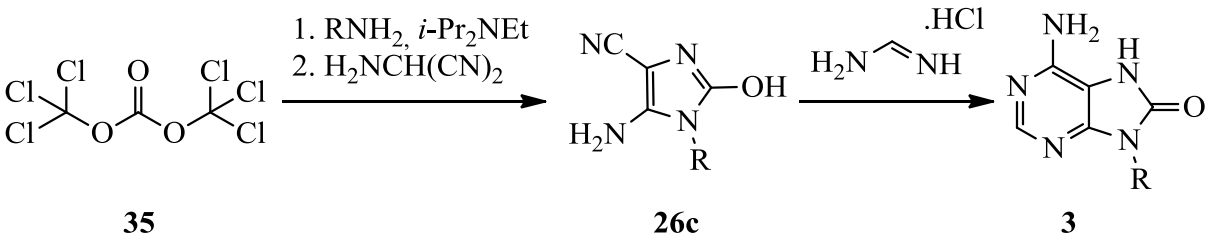
Scheme 16. Assumed mechanism of base-catalysed hydrolysis of 9-substituted-8-haloadenines (**6** or **8**) to give 9-substituted-8-oxoadenines (**3**).

CHAPTER 3

3. SYNTHESIS OF TARGET MOLECULES

3.1. General

The synthesis of the target molecules presented some challenges and, despite the structural similarity of these compounds, a variety of synthetic methods were used to obtain the final compounds. Compounds **3a**, **3b** and **3d** have been synthesised earlier by a different route and for other purposes and this method is shown below (Scheme 17).[55] That route involved the creation of a 1-substituted imidazole ring (**26c**) using triphosgene (**35**), the relevant primary amine and aminomalononitrile.[55,56] The pyrimidine ring was then closed using formamidine hydrochloride to give **3**. The yields for these steps are presented in Table 1.



35 **26c** **3**

Scheme 17. Previously published method of synthesising 9-substituted 8-oxoadenines (**3**).

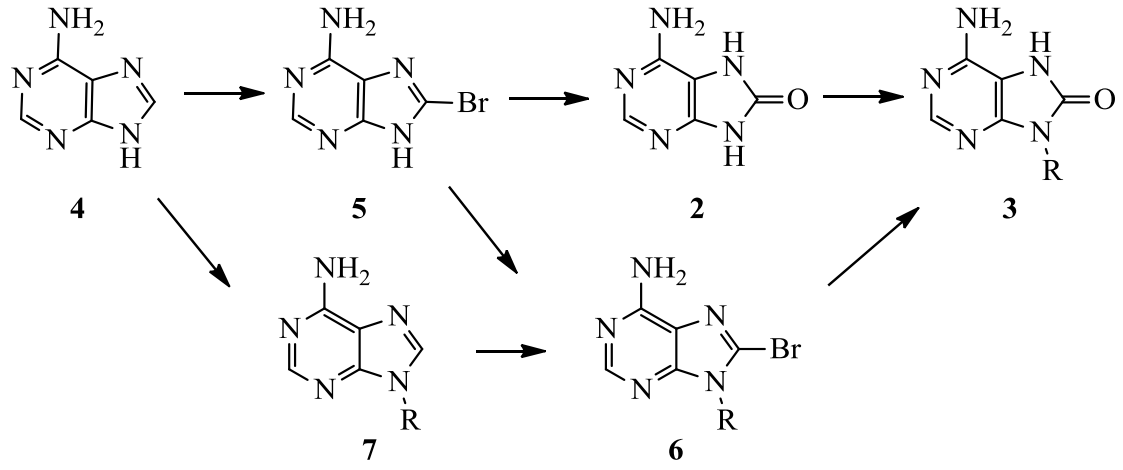
Table 1. Summary of relevant results from the known synthesis route.[55,56]

Entry	R	Imidazole step [%]	Pyrimidine step [%]	Overall yield [%]
1	**a:** Benzyl	85	80	68
2	**b:** Cyclopentyl	No information given		
3	**d:** Phenyl	73	74	53

This reaction is elegant on paper, but it should be noted that although triphosgene is claimed to be safer than phosgene and diphosgene, its vapour phase is still reported to be toxic. In addition, the first two steps of this reaction to create the imidazole ring involve the evolution of two equivalents of phosgene gas to each mole of product created. Without having first-hand experience of this reagent, it seemed to have valid safety concerns.

With this in mind, a new strategy for obtaining the target molecules was designed. By considering the structure of the target molecules, it could be seen that, starting from purine, there are three functional groups that are attached to the main bicyclic structure. The amine group on C-6 could easily be obtained by using commercially-available adenine as a starting point. The oxo group on C-8 could be established in two steps through halogenation and subsequent hydrolysis. The R group on *N*-9 could be attached through *N*-alkylation or *N*-arylation.

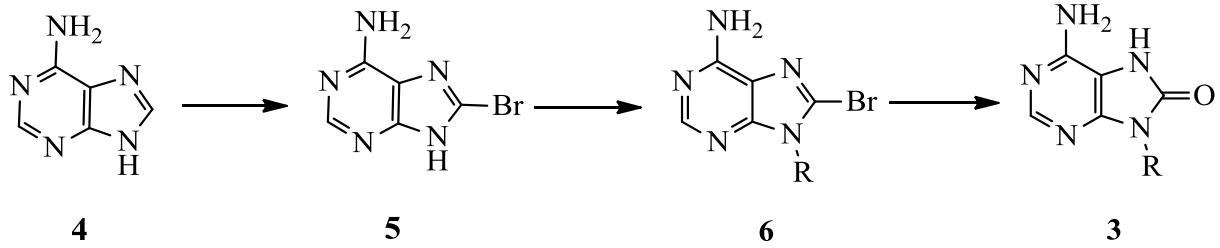
There are therefore three transformations that must be carried out starting from adenine to synthesise the target molecules and three synthetic pathways can be imagined to accomplish these transformations (Scheme 18). In the interests of reaction economy, it would be logical to introduce the different side-groups as late as possible (Route compounds **4** to **5** to **2** to **3**). However, alkylation of compound **2** appeared more complicated than alkylation of compounds **5** based on similar reported reactions,[57] with not only the question of regioselectivity, but also dialkylation. It was thus seen to be best to attempt first alkylation of **5** – which should only give monoalkylated products,[28] then hydrolyse to **3**.



Scheme 18. The initial possible routes for synthesising the target molecules (**3**).

3.2. Strategy 1 – Bromination, Alkylation and Hydrolysis

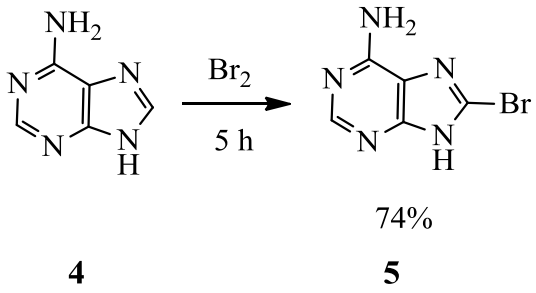
The synthetic route for the target compounds, 9-substituted 8-oxo-9*H*-purin-6-amines (**3**), is outlined in Scheme 19. The initial approach to obtain the target molecules was to brominate adenine (**4**) at the C-8 position and then alkylate the resulting compound **5** with the relevant alkylating at *N*-9. The result was expected to be a mixture of 9- and 3-regioisomers. This mixture of isomers would then be separated by flash chromatography and the desired *N*-9 isomer (**6**) was to undergo hydrolysis to obtain the target molecules (**3**).



Scheme 19. Route for bromination, benzylation and hydrolysis.

3.2.1. Bromination of Adenine

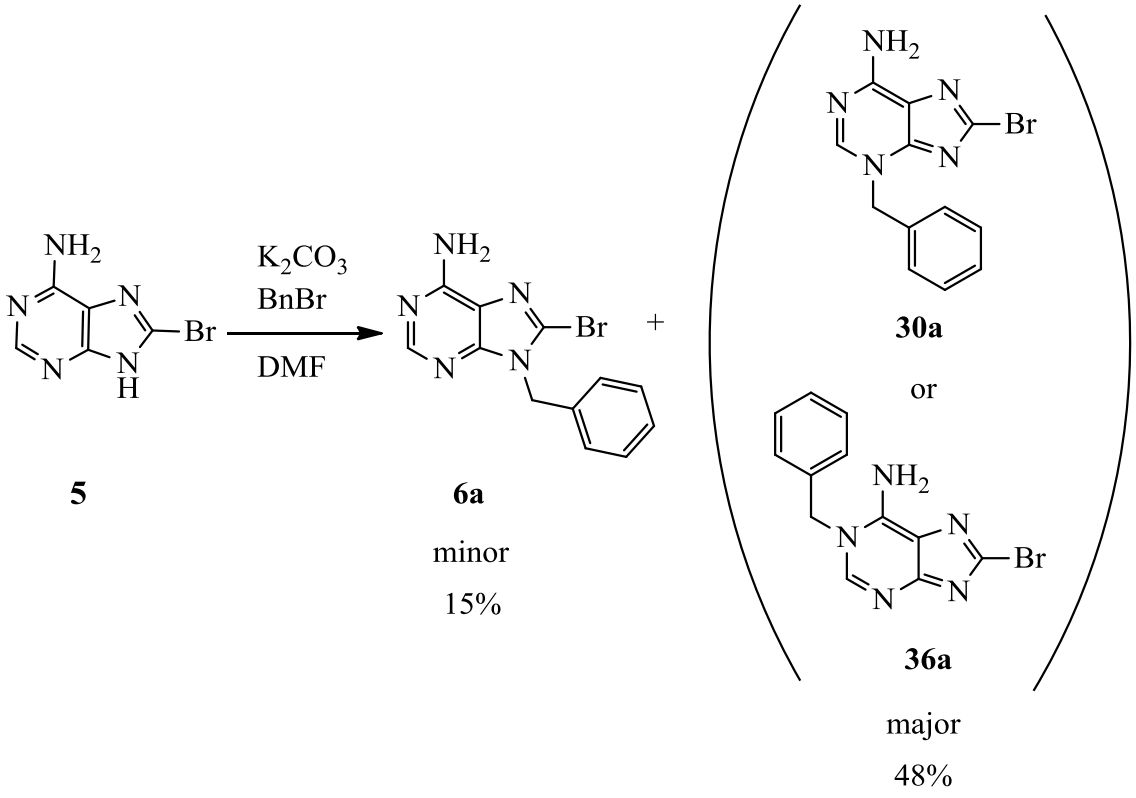
Bromination of adenine was achieved through a literature procedure by treating adenine (**4**) with liquid bromine at ambient temperature for 5 h (Scheme 20).[58,59] After neutralisation, washing and drying, the result was the desired product (**5**) as a beige powder in a good 74% yield.



Scheme 20. Bromination of adenine (**4**) using liquid bromine.

3.2.2. Alkylation of 8-Bromoadenine with Benzyl Bromide

9-Alkylation of 8-bromoadenine (**5**) with benzyl bromide to prepare compound **6a** was attempted by generally following the procedure used for 9-alkylation of 8-bromoadenine with 4-bromobutyl acetate in the presence of potassium carbonate, which involved heating the reaction mixture at 135 °C for 8 h, which is reported to give 45% 9-substituted and 22% 3-substituted product.[58] Benzylation using benzyl chloride instead of benzyl bromide has been reported, but the yields are lower (32% and 15% for 9- and 3-substituted, respectively).[60] Based on the reported yields, the first method was pursued for the current project (see Scheme 21).



Scheme 21. General scheme for alkylation of 8-bromoadenine (**5**) under basic conditions.

It was decided to try this reaction at the 135 °C overnight. Unfortunately, these conditions resulted in many products that were inseparable *via* flash chromatography (Table 2, Entry 1). The procedure was also carried out at a lower reaction temperature (ambient temperature), unfortunately giving similar results (Entry 2). By repeating the first reaction and tracking the reaction progress *via* TLC, it was observed that multiple spots started appearing after 4 hours

(Entry 3). The most successful results obtained in the current project were accomplished by both shortening the reaction time and using ambient temperature. Conducted at ambient temperature for 4 h (Entry 4), the reaction gave two monoalkylated products (**6a** and a monoalkylated by-product, **30a** or **36a**, see Scheme 21) and a small amount of dialkylated product (not isolated pure), all running close on silica TLC in various eluents.

Table 2. Summary of results from ^{1}H NMR spectra of the crude reaction mixtures from experiments during optimisation of the synthesis shown in Scheme 21.

Entry	**5 [mmol]**	**K_2CO_3 [eq]**	**T [°C]**	**t [h]**	**DMF [mL]**	**Crude NMR**
1	1	2	135	16	5	Multiple purine and benzyl CH_2 peaks
2	1	2	r.t.	16	5	Multiple purine and benzyl CH_2 peaks
3	1	2	135	4	5	Fewer purine and benzyl CH_2 peaks than for Entries 3 and 6
4	1	2	r.t.	4	5	1:2 **6a**:**30a/36a**
5	0.5	2	40	4	5	1:2 **6a**:**30a/36a** and additional purine and benzylic methylene peaks
6	0.5	2	0	4	5	1:9 **6a**:**30a/36a**

To see if further change in temperature could improve regioselectivity, small-scale reactions were carried out at 40 °C and 0 °C (Entries 5 and 6, respectively). The first increased the number of products produced in the reaction while lowering the temperature to 0 °C appeared to favour the undesired by-product. These conclusions are drawn from a combination of TLC and inspection of the ^{1}H NMR spectrum of the crude mixture for these reactions, by comparing the integral values of the benzylic methylene signals. The shifts for these signals had been obtained in previous attempts and have also been reported in literature.[60]

9-Benzyl-8-bromo-9*H*-purin-6-amine (**6a**) was finally obtained by treating 8-bromoadenine (**5**) with benzyl bromide in the presence of a potassium carbonate for 4 h (Scheme 21). Unfortunately, this method gave a low yield of 15% for the desired product and a much higher yield of 42% for the by-product (**30a** or **36a**).

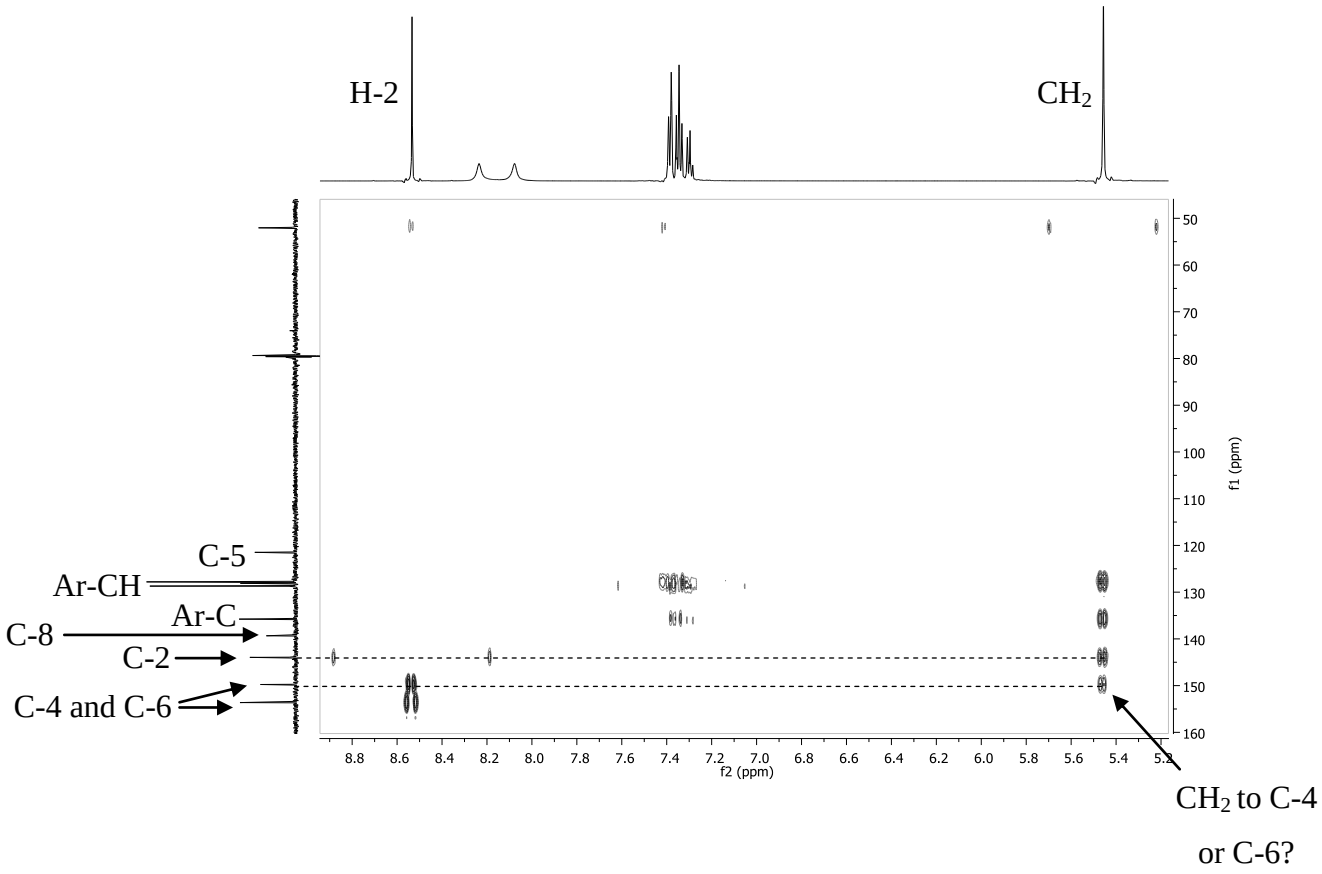
Based on reported alkylations and arylations of 8-bromoadenine, the main product was expected to be the 3-alkylated regioisomer since alkylation of 8-bromoadenine under basic conditions is reported to often give mixtures of the 3- and 9-alkylated isomers.[42,57-61]

However, when it came to characterisation of this compound, the position of the benzyl group was unable to be ascertained with certainty by NMR techniques and could be either the 1-benzylated (**36a**) or 3-benzylated (**30a**) isomer (see Scheme 21). Furthermore, the ^{1}H NMR spectrum of the same compound showed some intriguing features discussed below, and hence NMR and X-ray structural studies were carried out on this compound.

3.2.2.1. NMR Spectroscopy Investigations

The spectral data for the major product (**30a** or **36a**) were in good agreement with what has been reported for compound **30a** before, but in the literature, structure elucidation is claimed to be based on ^{1}H-^{13}C HMQC and HMBC NMR (no details given).[60] However, despite both the proton and carbon spectra for our product being in good agreement with the literature data, the HMBC data we obtained were inconclusive.

The CH_2 protons correlate to two carbon shifts; the C-2 at 143.9 ppm and a peak at 149.8 ppm (quaternary C). It was not possible to determine of the latter peak was the C-4 or C-6 shift and hence it was not possible to determine if the benzyl group was situated at *N*-3 or *N*-1. Both the peak at 149.9 and a second peak (quaternary C) at 153.6 ppm correlated to H-2 and neither correlated with the NH_2 in the HMBC spectrum (see Spectrum 1 and Figure 14 below).



Spectrum 1. HMBC spectrum of the major isomer formed from the reaction shown in Scheme 21 with the problem indicated.

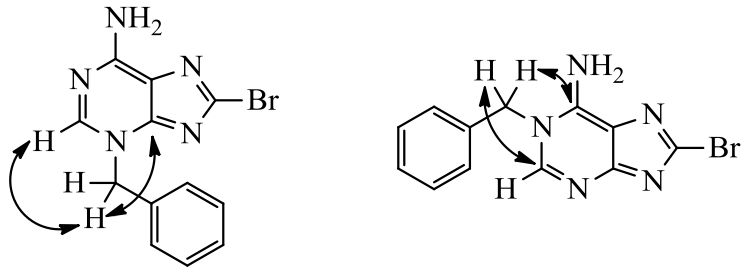


Figure 14. The possible structures of the two isomers that are in agreement with the relevant and possible HMBC correlations.

Several reports have been published on the formation of *N*-3 alkylated 8-bromoadenine, where the structure elucidation is reported to be based on ^{1}H-^{13}C HMQC and HMBC NMR.[42,59,60] As our NMR studies were inconclusive, we decided to determine with absolute certainty whether the major product formed was the isomer **30a** or **36a**. For these purposes, we turned to X-ray crystallography.

3.2.2.2. X-ray Crystallography Investigations

Prior to this investigation, crystal structures of ten 8-bromoadenines were available in the Cambridge Structural Database (CSD, Version 5.31 of November 2009).[62] However, all these molecules are 9-substituted. No 1- or 3- substituted 8-bromoadenines are previously reported in the database.

The result of the X-ray structural investigation shown in Figure 15, confirms that isomer **30a** has been crystallised. This is not only the first ever X-ray structure of an *N*-3 functionalised 8-bromoadenine, but in fact also the first X-ray structure of an *N*-3 functionalised adenine where neither *N*-7 nor *N*-9 act as ligands for metal ions or carry additional functional groups.

The asymmetric unit consists of two rotamers of compound **30a**. Both forms correspond to the amino tautomer and both amino H-atoms were easily located in the electron density map and participate in strong hydrogen bonds as shown in Figure 16. Bond lengths and bond angles are roughly the same for the two forms.

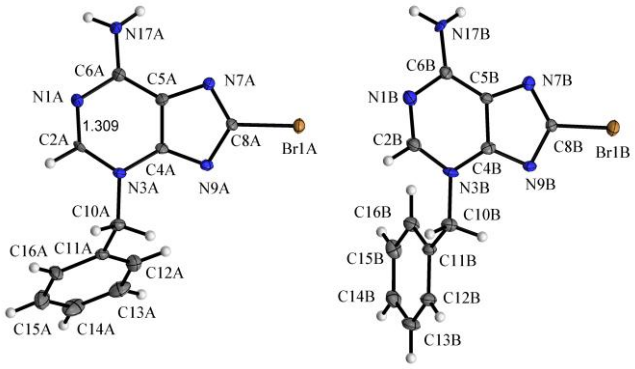


Figure 15. The two adenine molecules in the asymmetric unit of compound **30a** with atomic numbering indicated (not in correct crystallographic positions relative to each other). Displacement ellipsoids are drawn at the 50% probability level; H atoms are spheres of arbitrary size. Important bond lengths (in Å) have been indicated, estimated standard deviations are 0.005 - 0.006 Å. The different orientations of the benzyl groups are defined by the torsion angles C2A-N3A-C10A-C11A = 70.6(6)°, N3A-C10A-C11A-C12A = 76.7(6)°, C2B-N3B-C10B-C11B = 90.8(6)° and N3B-C10B-C11B-C12B = 177.7(5)°.

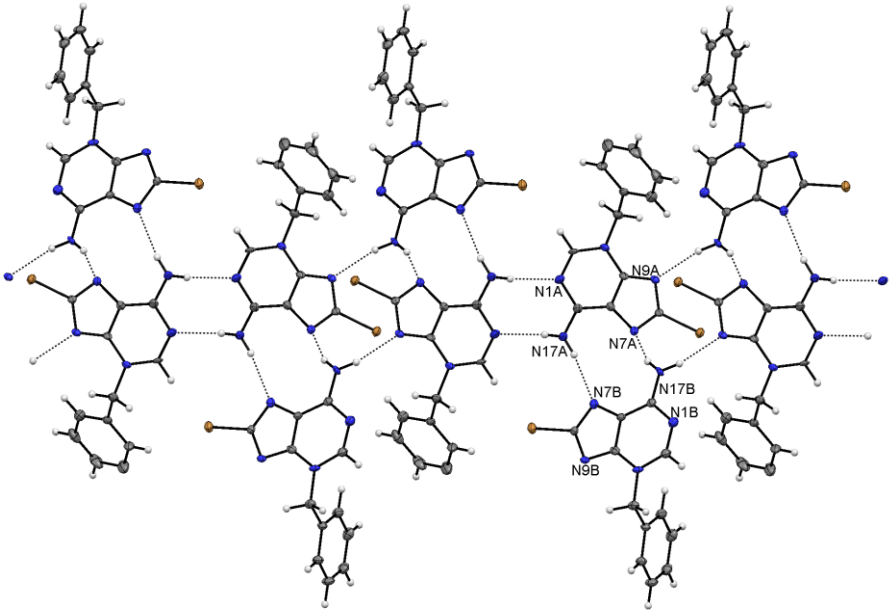
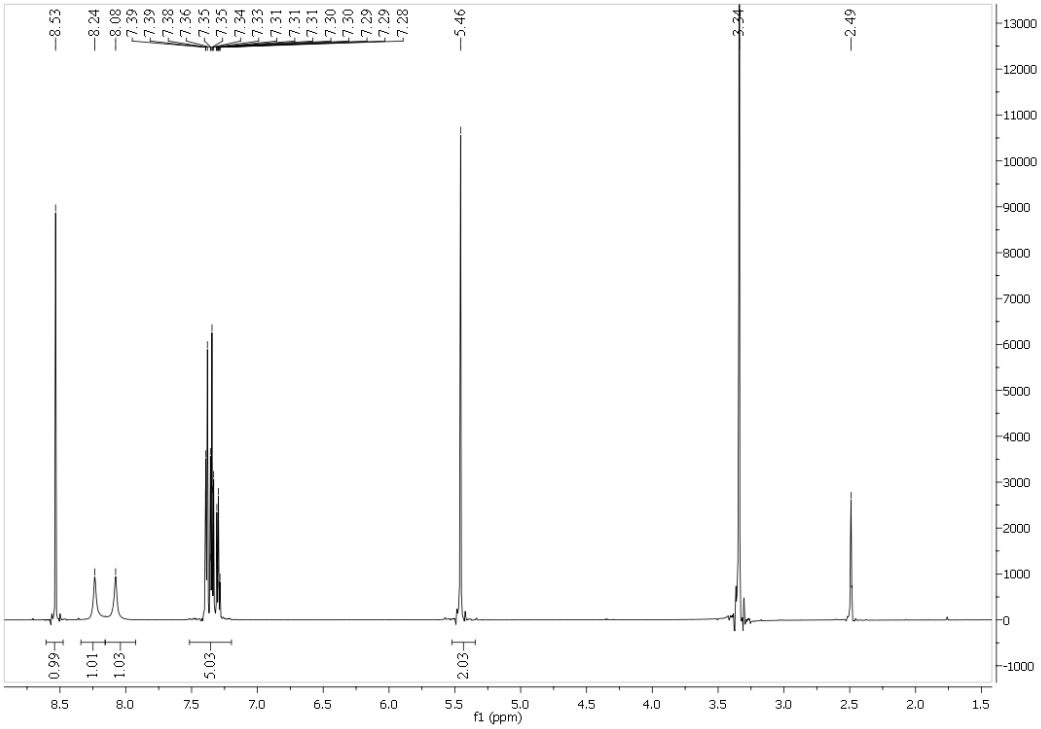


Figure 16. Adenine molecules of compound **30a** connected by hydrogen bonds into one-dimensional chains or tapes. The indicated H···N distances are in the range 2.09(4) - 2.16(3) Å. It can be seen that molecule A participates in a larger number of strong interactions as the aromatic N atoms accept a total of three H atoms compared to only one H atom for molecule B.

3.2.2.3. Tautomeric Considerations

In the ^{1}H NMR spectrum of compound **30a** in DMSO-d_6 solution, there are two distinct NH signals (8.07 and 8.23 ppm), in contrast to the spectrum of the 9-benzylated product **6a** where one broad singlet for the NH_2-group is observed (see Spectrum 2 below). Two NH signals have also been observed in spectra of other 3-alkylated adenines,[57,59-61] but this phenomenon is only discussed in one publication and the hypothesis presented is that the compound studied must exist in solution as an imine tautomer, but no experimental evidence is given.[61]



Spectrum 2. ^{1}H spectra of 3-benzyl-8-bromoadenine (**30a**).

It was hypothesised that the reason for the splitting of the NH signals could either be that compound **30a** exists in solution as an imine tautomer **37a** or **37b** (Figure 17) or that there is a restricted rotation around the N^6-C-6 bond leading to two resonances for the NH_a and NH_b as they are chemically different as seen for resonance forms **37c** or **37d** (Figure 18). The fact that calculations indicate that the amino tautomer of 3-methyladenine is more stable than any of the possible imine forms in the gas phase,[63] supports the latter hypothesis shown in Figure 18.

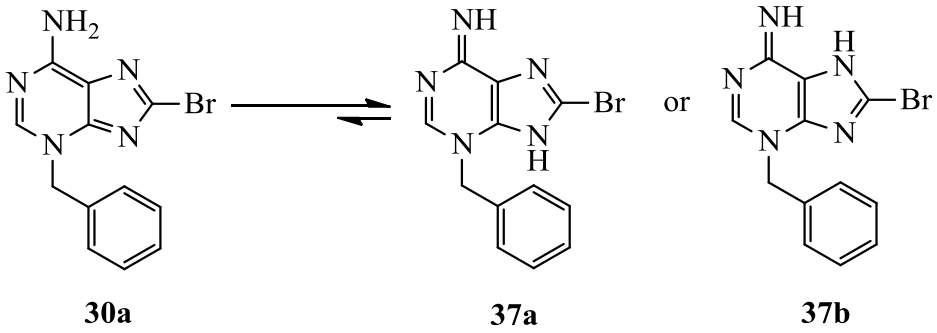


Figure 17. Possible tautomers **37a** and **37b** of compound **30a** in solution.

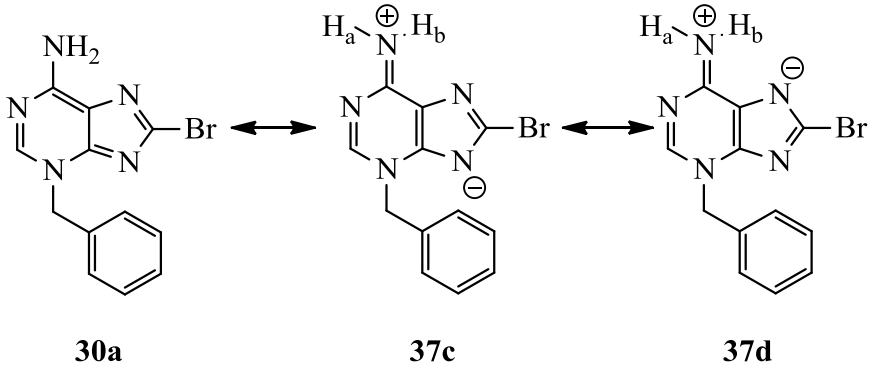


Figure 18. Three resonance forms **37c** and **37d** that may contribute to the structure of **30a**.

^{1}H-^{15}N HSQC NMR spectroscopy showed that both NH signals correlated to the same nitrogen signal at 125.7 ppm (relative to $^{15}NH_3$), proving that compound **30a** exists as the amino tautomer in solution (DMSO-d_6), with **37c** and/or **37d** contributing to the difference in chemical environment of the two NH protons.

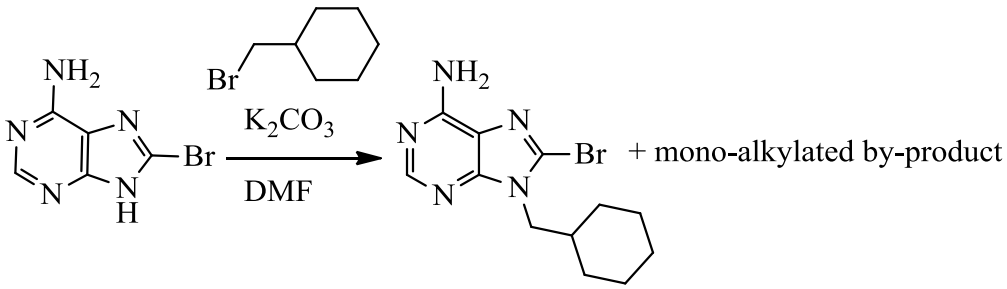
It is interesting to note that the NH_2 in compound **30a** is shifted substantially downfield compared to for instance the NH_2 in 9-methyladenine which resonances at 79.6 ppm (-300.9 ppm relative to $Me^{15}NO_2$)[64] indicating more sp^2 character for the N^6 in compound **30a**. This supports the explanation for the splitting of the NH signals based on hindered rotation of the C-N bond. The coalescence temperature for the NH_a and NH_b protons was not determined, but at 65 ^{o}C only one broad signal from the NH_2 could be seen in the ^{1}H NMR spectrum.

3.2.2.4. Conclusions of this Study

In summary, we have shown with absolute certainty that benzylation of 8-bromoadenine in the presence of K_2CO_3 gives a mixture of the *N*-3 and the *N*-9 alkylated isomers, with the former as the major product. The *N*-3 selectivity is higher when the reaction is conducted at ambient temperature compared to elevated temperatures. Furthermore, it is shown that the 3-benzyl adenine derivative exists as the amine tautomer in both the crystalline state as well as in solution (DMSO-d_6), but with restricted rotation around the N^6-C-6 bond. The results of this study have been submitted and accepted for publication in the Journal of Heterocyclic Chemistry (see Author Proof in Appendix 1).[65]

3.2.3. Alkylation of 8-Bromoadenine with (Cyclohexyl)methyl Bromide

Despite the results from benzylation of 8-bromoadenine, the same procedure was attempted to synthesise 9-(cyclohexylmethyl)-8-oxo-*9H*-purin-6-amine (**6c**). The alkylation step (Scheme 22) proved equally, if not more challenging than the benzylation of the same substrate. The experiment was followed on TLC over 4.5 h in the first attempt and run over 1 day in the second attempt.



5 **6c**

Scheme 22. Alkylation of 8-bromoadenine (**5**) with (cyclohexyl)methyl bromide.

Examination of the ^{1}H NMR spectra of the crude mixtures of these experiments showed that the overall conversion from starting material to two mono-alkylated products was nearly identical (37% and 38%, respectively) and the ratio of 9-alkylated to another mono-alkylated product was similar (4:3 and 1:1, respectively) (Table 2). The 9-alkylated product (**6c**) and by-

product (not isolated in pure form) ran very close on TLC using various eluents and separation by column chromatography gave only 14% of the desired product pure and impure fractions of the by-product. The structure of the 3-alkylated product was postulated by comparison of the ^{1}H NMR spectrum and retention factor values with those from the benzylation reaction). The results of this alkylation were not satisfactory and the amount of 9-alkylated isolated was too small to attempt the next step.

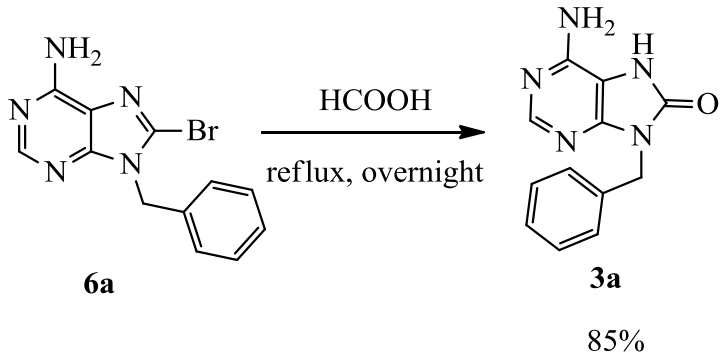
Table 3. Calculated yield of starting material, 9-alkylated and 3-alkylated in crude product, determined from ^{1}H NMR spectra of the crude mixtures, from the reaction shown in Scheme 22.

Entry	**t [h]**	**Unreacted starting Material [%]**	***N*-9 alkylated [%]**	***N*-3 alkylated [%]**
1	4.5	63	21	16
2	24	61	19	19

3.2.4. Hydrolysis of Monoalkylated-8-Bromoadenines

There are several known methods for carrying out hydrolysis of a bromo group to an oxo group in an adenine compound. One way is to treat the 8-bromoadenine derivatives with sodium acetate in acetic acid under reflux for 8 h.[57] An alternative method is by heating the substrate at reflux in 1M aqueous sodium hydroxide for 90 min.[66] The chosen method was a third alternative, using formic acid at high temperature to obtain the hydrolysed product.[54]

Compound **6a** was thus hydrolysed to the desired target molecule, 6-amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**), by heating at reflux in formic acid overnight and purified by flash chromatography, resulting in 85% yield (Scheme 23).

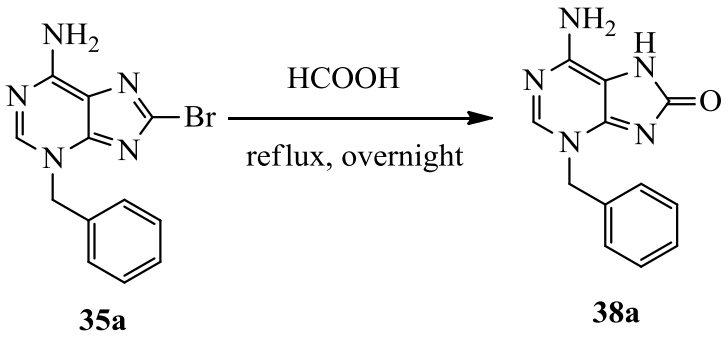


6a **3a**

85%

Scheme 23. Hydrolysis of 9-benzyl-8-bromoadenine (**6a**) with formic acid.

The 3-benzylated isomer (**30a**) was also hydrolysed using the same reaction conditions to give 33 % of to 6-amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**) after recrystallization (Scheme 24).



35a **38a**

33%

Scheme 24. Hydrolysis of 3-benzyl-8-bromoadenine (**30a**) with formic acid.

The lower yield of 33% for the hydrolysis of compound **30a** is possibly a reflection of the purification method used. At first, both the 9- and 3-oxoadenines (**3a** and **38a**) were purified by recrystallization from chloroform/methanol with 37% and 33% yields, respectively. However, it was subsequently discovered that carrying out flash chromatography on the crude mixture from the former substrate gave a high yield of 85%. Hydrolysis of compound **30a** was not repeated to test the effect on purification methods, because this compound was not a target molecule.

3.2.5. Conclusions

The described strategy consists of three steps where the first and last (based on one substrate) allow good yields. The major disadvantage of the strategy is the second step of *N*-9 alkylation. The main problem for the second step was the low conversion to the desired product. In addition, the monoalkylated-8-bromoadenine compounds are very polar and this resulted in low solubility on the flash column. This factor, in combination of with similar R_f-values and the tailing effect brought on by the presence of the amino-group on C-6, resulted in very difficult separations during flash chromatography, with many mixed fractions and only a small percentage of the desired products isolated in pure form. The overall yield for the synthesis of compound **3a** using Strategy 1 was 9%.

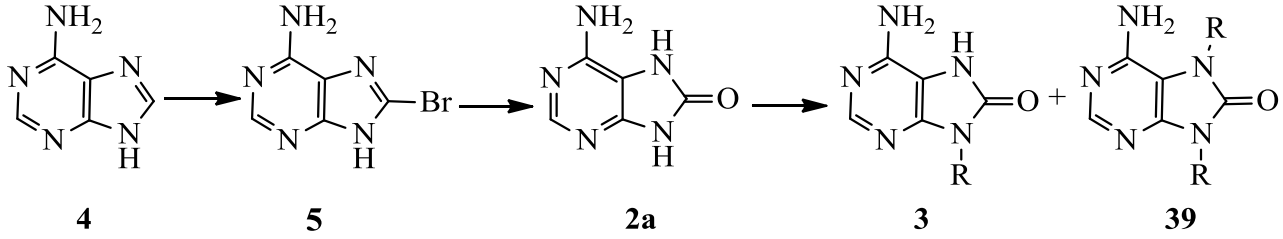
It is interesting to note that the ratio of the isomeric products is different for the reaction with benzyl bromide and those with (cyclohexyl)methyl bromide. Possible factors may be sterical effects from the halide, electronic effects, and the identity of the alkylating agent or base, among other factors. It could be interesting to carry out a survey of the alkylating patterns of 8-substituted adenines at some time. Further research toward this may provide useful information in functionalization of adenines.

Because of the poor results in the second step of the synthetic pathway, it was seen as undesirable to pursue further optimisation or to attempt this strategy with other 9-substituents.

3.3. Strategy 2 – Bromination, Hydrolysis and Alkylation

3.3.1. General

Since the initial approach (see Section 3.2) did not give as good results as had been hoped, it was decided to attempt a different approach to the synthesis to hopefully improve yields and separation. Carrying out the hydrolysis of the 8-bromo group before introduction of the side-group on *N*-9 could result in easy access to a range of 9-substituted 8-oxoadenines (**3**) and reaction economy with fewer reactions to reach multiple analogues of compound **3** (Scheme 25).

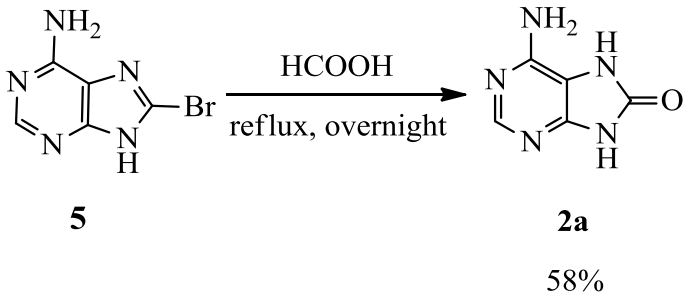


Scheme 25. Alternative synthesis of target compounds **3** *via* bromination, hydrolysis and alkylation.

3.3.2. Hydrolysis of 8-Bromoadenine

Hydrolysis of 8-bromoadenine (**5**) has been reported twice in literature – one with the use of acetic acid and once with formic acid.[67,68] We decided to use formic acid to keep the step the same as the hydrolysis step in Strategy 1, especially since literature reported similar yields of 83% and 88%, respectively, for the two procedures.

8-Bromoadenine (**5**), which had been synthesised as described in Section 3.1, was thus hydrolysed by heating 8-bromoadenine at reflux in formic acid overnight. Recrystallization from water gave the product in a 58% yield (Scheme 26). The NMR data was in good agreement with the literature.[57,67]



5 **2a**

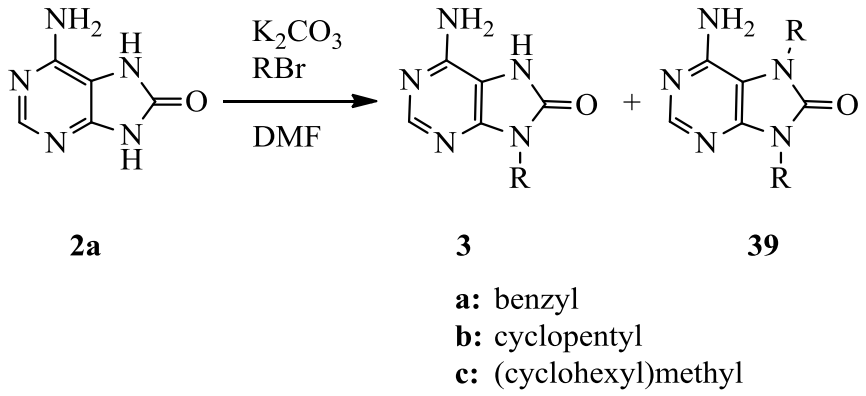
58%

Scheme 26. Hydrolysis of 8-bromoadenine (**5**) to 8-oxoadenine (**2a**).

3.3.3. Alkylation of 8-Oxoadenine

Alkylation of 8-oxoadenine has been reported once previously.[57] In that procedure, 8-oxoadenine was treated with a sodium hydride and the alkylating agent added after 1 h at 110 ºC. The mixtures were then stirred at the same temperature for a period varying between 4 h and 25 h. The results were mixed with reported yields varying for each alkylating agent. Yields of the monoalkylated products ranged from 29% to 55% and yields of the dialkylated products ranged from 20% to 34%.[57]

It is reported that ratio of the mono- and dialkylated products is affected by the excess of alkylating agent (more alkylating agent is observed to give more dialkylated product).[57] The amount of alkylating agent was therefore kept low – 1.2 equivalents. Since we considered that a weaker base should be sufficient to deprotonate at least one proton on *N*-9 or *N*-7, it was decided to use potassium carbonate instead of sodium hydroxide. In addition, it was decided to begin at ambient temperature and increase the temperature if necessary. Compound **2** was then reacted with three alkylating agents – benzyl bromide, (cyclohexyl)methyl bromide and cyclopentyl bromide.



Scheme 27. Reaction of 8-oxoadenine (**2a**) with alkyl bromides under basic conditions.

3.3.3.1. Alkylation of 8-Oxoadenine with Benzyl Bromide

Alkylation of 8-oxoadenine (**2a**) with benzyl bromide was initially planned to be carried out under the same conditions as was previously done for the alkylation of 8-bromoadenine (**5**). However, the reaction time was reduced from 4 h to 3 h because additional by-products appeared to be forming as judged from TLC taken during the reaction period. This method gave 19% (36 mg) 9-benzyl-8-oxoadenine (**3a**) and the 7,9-dialkylated product (**39a**) (~30%, impure). The separation of the products was significantly better than that presented in Section 3.1 even though the conversion and yield was not improved.

3.3.3.2. Alkylation of 8-Oxoadenine with (Cyclohexyl)methyl Bromide

For reaction of compound **2a** with (cyclohexyl)methyl bromide to give compound **3c**, the conversion rate in the alkylation step proved much slower than in the case of benzylation of the same substrate. This was not unexpected since (cyclohexyl)methyl bromide was also observed to be slower to react in the alkylation of 8-bromoadenine.

This difference could be explained by benzyl bromide being able to form a resonance-stabilised cation and undergo a more S_N1-type substitution whereas the cation intermediate of (cyclohexyl)methyl bromide would only be stabilised by inductive donation from the cyclohexyl group.[31] Of course, both substrates could undergo S_N2 substitution since they are primary alkyl halides,[31] so the difference may be that benzyl bromide reacts *via* a different mechanism.

As a result, the reaction mixture had to be warmed, in the first instance to 50 °C, then 70 °C in the second. In addition, the reaction time was increased to 21 h and 24 h, respectively. At 50 °C, the conversion was approximately 30% from crude NMR and appeared greater at 70 °C on inspection *via* TLC. However, crude NMR indicated that several purine compounds were being produced. Similar yields of 28% and 21%, respectively, were obtained with more starting material being recovered from the first instance. TLC indicated more dialkylated products being formed at the higher temperature and after flash chromatography; several mixed fractions indicating multiple dialkylated products were isolated (see Table 4).

Table 4. Results of alkylation of compound **2** with (cyclohexyl)methyl bromide.

Entry	**T [°C]**	**t [h]**	**Yield of *N*-9 alkylated (3c) [%]**	**Yield of 7,9-dialkylated (39c) [%]**
1	50	21	21	Not isolated
2	70	24	28	~3

The structure of the dialkylated compound isolated in the most pure fractions were indicated by 2D NMR to be the dialkylated structures shown in Scheme 27 – i.e. 7,9-dialkylated, as was also seen for the benzylation attempt.

3.3.3.3. Alkylation of 8-Oxoadenine with Cyclopentyl Bromide

Alkylation of 8-oxoadenine (**2a**) with cyclopentyl bromide was also carried out, at slightly lower temperature (40 ºC) and over a longer period of time than the initial conditions used for alkylation with (cyclohexyl)methyl- bromide to try to avoid the multiple dialkylated by-products seen in those alkylations. However, since cyclopentyl bromide is a secondary alkyl halide, it is more sterically hindered than either of the other alkylating agents and thus less accessible for nucleophilic attack.[31] After 2 days, there was observed only ~15% conversion by inspection of the ^{1}H NMR spectra of the crude mixtures. The temperature was raised slightly to 60 ºC and the conversion did not increase significantly, although TLC indicated more by-product formation.

When the reaction was stopped and worked up after 3 days at the higher temperature, there were three intense spots and several less intense spots visible on TLC. Crude NMR was not easy to interpret with several overlapping signals. The ratio of starting material to the two major products was estimated to be approximately (2:1.3:1 **2a**:**3b**:**39b**). After flash chromatography, the desired *N*-9-product was isolated in 20% yield and impure by-product (12%) also isolated.

3.3.4. Summary of the Alkylation of 8-Oxoadenine

The results of the alkylation of 8-oxoadenine (**2a**) are summarised in Table 5 below. The 9-monoalkylated compounds (**3**) were isolated in 15%, 28% and 27% yields, respectively. Impure 7,9-dialkylated products were also isolated in approximately 30%, 3% and 12% yields in addition to the observation of various other by-products in all three cases.

The impurities in the reactions with benzyl bromide and cyclopentyl bromide are assumed to be another dialkylated purine (possibly N^6, *N*-9), whereas DMF is the impurity in the reaction with (cyclohexyl)methyl bromide. The literature procedure mentioned in Section 3.3.3 involved the use of preparative chromatography on silica gel plates followed by recrystallization. That method may have given pure dialkylated product but on the scale carried out in this project, this was not practical. If the results had been promising, up-scaling would have allowed further purification of the by-product.

Table 5. Summary of the conditions and results obtained from alkylation of compound **2a** as shown in Scheme 25, indicating the yield of products and recovered starting material (R.S.M.).

Entry	**R**	**t [ºC]**	**T [h]**	**Yield of monoalkylated [%]**	**Yield of dialkylated [%]**	**R.S.M. [%]**
1	**a:** Benzyl	r.t.	3	19	~30 (impure)	-
2	**c:** Cyclopentyl	40 60	48 72	27	~12 (impure)	27
3	**b:** (Cyclohexyl)methyl	50	21	28	~3 (impure)	60

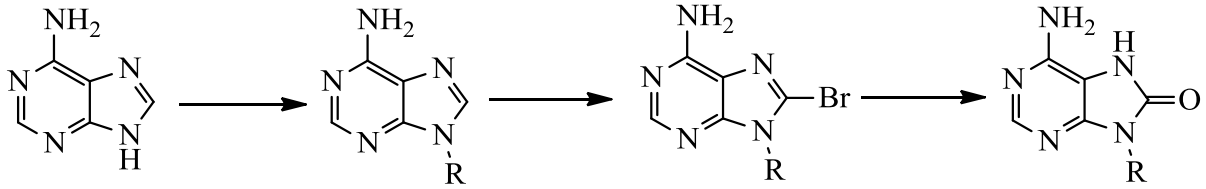
3.3.5. Conclusions

Steps 1 and 2 of this synthesis route worked well with moderate yields. However, the third step gave poor results and it appeared that this method was also not the ideal method of producing *N*-9-alkylated products (**3**) in high yields. The conditions in the third step produced a variety of dialkylated products (**39**), which resulted in a less straightforward purification *via* flash chromatography than had been expected. In addition, it seemed that raising the temperature to increase the rate of conversion resulted in the production of more undesired dialkylated product. The overall yields were 6%, 12% and 12% for **3a**, **3b** and **3c**, respectively.

3.4. Strategy 3 – Alkylation, Bromination, Hydrolysis

3.4.1. General

An alternative approach to the synthesis of the target molecules was to reverse the first two steps shown in Scheme 19 above and N-alkylate adenine (**4**) before bromination at C-8 (see Scheme 28). The resulting 9-substituted 8-bromoadenines (**6**) could then be hydrolysed in the manner shown in earlier in Scheme 23 to give the target compounds (**3**).

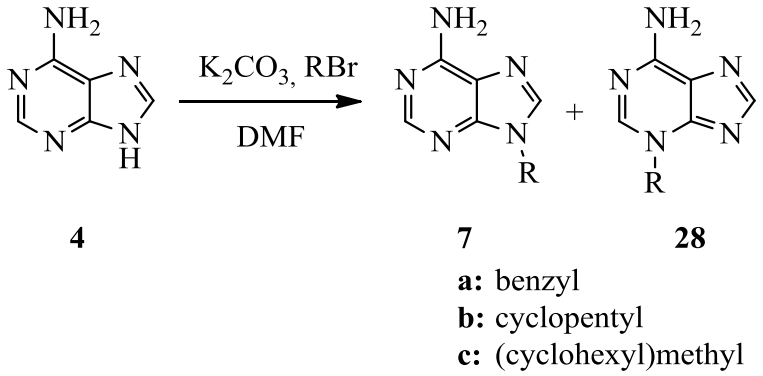


4 **7** **6** **3**

Scheme 28. Third possible route for synthesis of 9-substituted-8-oxoadenines (**3**).

3.4.2. Alkylation of Adenine

N-Alkylation of adenine (**4**) was carried out through a literature procedure where adenine was reacted with an alkyl halide in dry DMF employing potassium carbonate as a base (Scheme 29).[34] Using benzyl bromide as the alkylating agent, the reaction time used was 4 h while for reactions using the less reactive (cyclohexyl)methyl bromide and cyclopentyl bromide a reaction time of 72 h was required for complete conversion (Table 6).



Scheme 29. Alkylation of adenine (**4**) using alkyl halides under basic conditions.

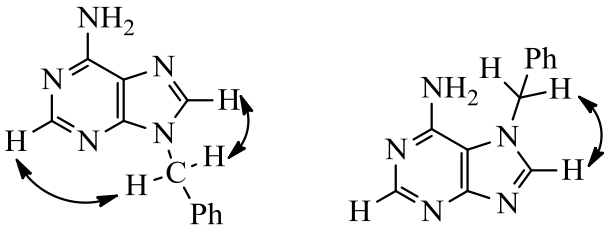
According to literature, in some of these reactions the *N*-7 isomer has been found to be produced in addition to the *N*-9, although in much lower yields (9%-27%).[34] However, examination of NMR data obtained in the course of this project was not in agreement with these claims. The major products obtained by us were indeed the 9-alkylated isomers (**7**), but the minor products were discovered through 2D NMR experiments to be the 3-alkylated (**28**) and not the 7-alkylated adenines (**29**). The results of the alkylation step are shown below in Table 6. A brief discussion of the characterisation of the by-product follows.

Table 6. Results of alkylation of adenine (**4**) to give compounds **7a-c** and **28a-c** (isolated yields).

Entry	**R**	**Yield of *N*-9 [%]**	**Yield of *N*-3 [%]**
1	**a:** Benzyl	53	23
2	**b:** Cyclopentyl	70	7
3	**c:** (Cyclohexyl)methyl	71	13

3.4.2.1. Characterization of the By-product

In literature, the assignment of the minor product as the 7-alkylated isomer was achieved by selective irradiation of the hydrogens on the carbon closest to *N*-7. In this case, the benzyl-methylene atom in both products was irradiated. For the *N*-9-benzylated adenine (**7a**), a NOE correlation to both the purine CH signals (H-2 and H-8) was seen. For the by-product (reported to be *N*-7 alkylated, **29a**), only one correlation was visible (see Figure 19). According to the article, the by-product was thus confirmed through NOE difference spectroscopy to be 7-alkylated (**29a**).[34]

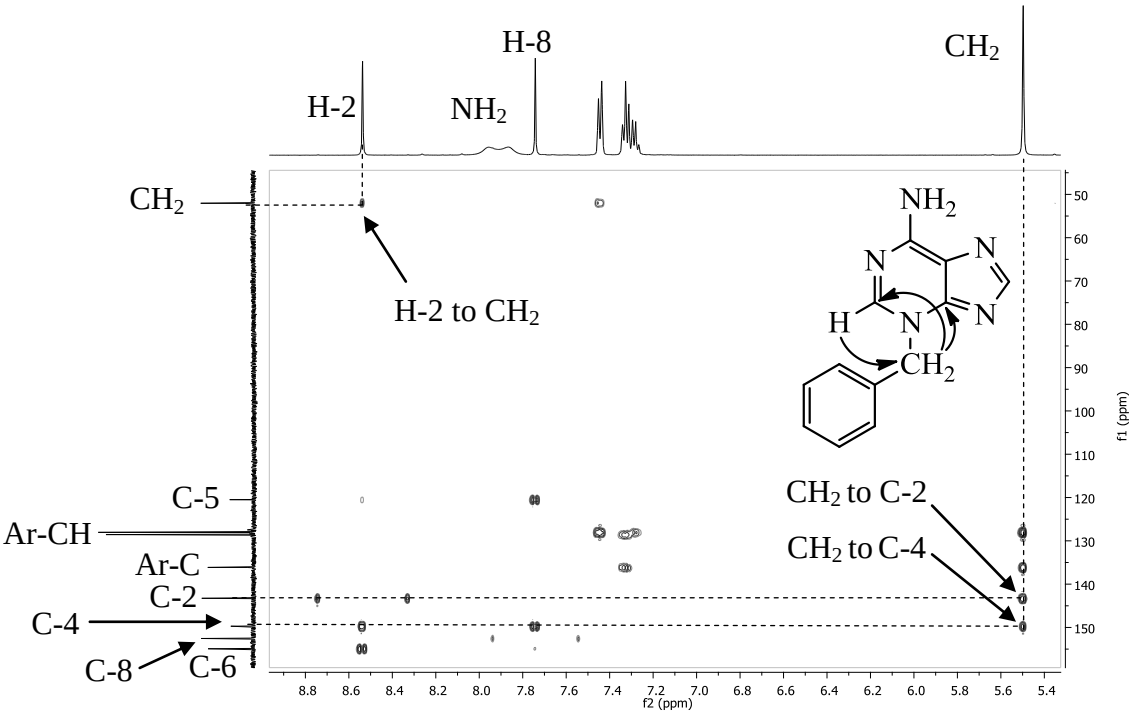


7a **29a**

Figure 19. Diagram indicating the expected NOE correlations for the *N*-9 (**7a**) and *N*-7 (**29a**) benzylated adenines.

In an article published earlier by the same authors, this structure elucidation by NOE difference spectroscopy for the same compounds is explained in more detail.[35] It is claimed that irradiation of the protons on the side chain of the by-product results in a correlation to one of the purine CHs and in addition, the exo-cyclic NH_2 group attached to C-6.

Interpretation of the 2D NMR data (HMBC and HSQC) data obtained in the course of the current project indicated a 3-subsituted isomer (see Spectrum 3), so it was thus decided to carry out 1D NOE experiments to see if the results obtained in literature could be reproduced.



Spectrum 3. HMBC spectrum and drawing of minor isomer indicating the relevant correlations.

The benzylated by-product was used as the test compound since the literature did not obtain any by-product when cyclopentyl bromide was used and did not synthesise the cyclohexylmethyl analogue. The methylene protons on the benzylic side chain were irradiated first, and then the closest phenylic protons were irradiated.

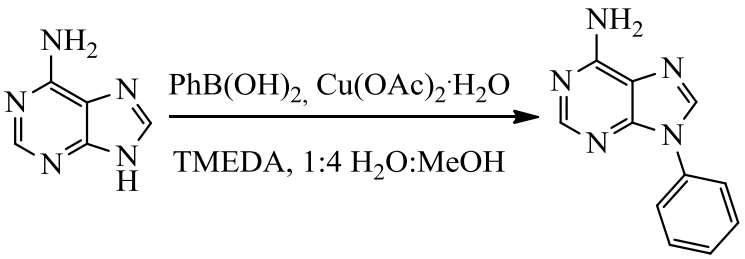
There was no visible correlation between the amino group on C-6 and any of the protons in the benzylic side-chain that should be closest to the groups if the benzyl group was located on *N*-7. Irradiation of the protons in the amino group gave no visible correlations. In addition, when irradiating the phenylic protons, a correlation was seen to H-2 and a very weak correlation to H-8, which seems unlikely to indicate *N*-7 alkylation but does not rule it out as a possibility.

Although the absence of NOE correlations is not proof of which regioisomer has been produced, the visible signals in the HMBC spectrum does indicate that, in our hands, alkylated of adenine results in the 3-alkylated isomer (**28a**) being obtained.

3.4.3. Arylation of Adenine

Arylation of adenine was planned to establish a phenyl ring on the 9-position. Previously, it had been reported that copper-catalysed coupling of phenyl boronic acid to adenine was unsuccessful in dichloromethane due to insolubility problems.[45] Subsequently, there have been several papers reporting the use of water/methanol and DMF as solvents with corresponding good to very good yields (85%[43,44] and 69%[42]) and it was decided to follow these procedures.

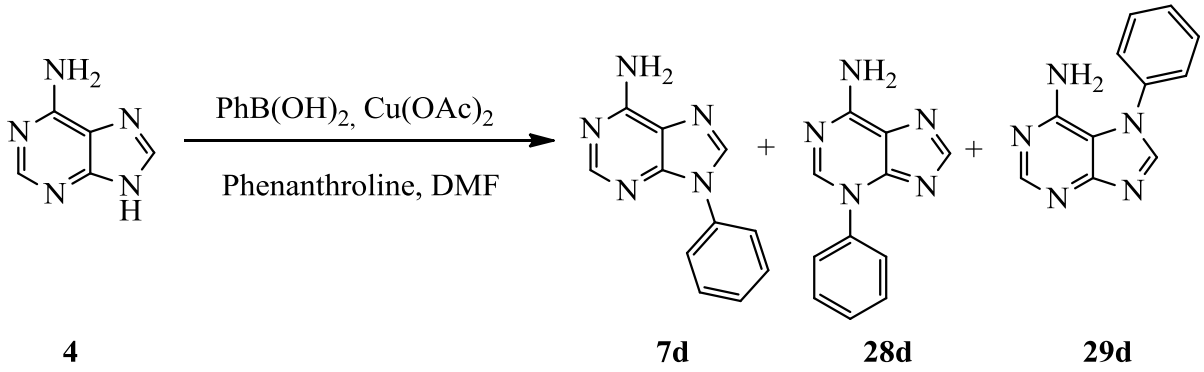
The first attempt at preparing 9-phenyl-9*H*-purin-6-amine (**7d**) involved the coupling of adenine (**4**) and phenyl boronic acid in the presence of a copper catalyst. Adenine (**4**) was first treated with phenylboronic acid in the presence of copper diacetate monohydrate and tetramethylethlyenediamine using a solvent system of water and methanol (Scheme 30). Unfortunately, this gave only low yields (13% after 45 mins, 20% after 22 h, literature yield = 85%).[43,44]



4 **7d**

Scheme 30. Attempted arylation of adenine.

The same arylation was attempted with phenanthroline as a ligand and DMF as a solvent (Scheme 31).[42] This procedure required the use of copper(II) acetate but did not specify if it was anhydrous or monohydrate. Since Yue,[44] had indicated that changing to copper(II) monohydrate gave better yields than using the anhydrous copper(II) acetate, this was attempted first. These conditions gave a 9-substitued (**7d**) pure in 21% yield (literature yield = 69%) and mixtures of 9-, 7- and 3-substituted product, identified by comparison of the ^{1}H NMR spectra with NMR data from literature (calculated overall yields = 23%, 19% and trace, respectively). Repeating the experiment with anhydrous copper(II) acetate gave 17% 9-substituted (**7d**) and ~25% 7-substituted (**29d**) (slight impurities) adenine.

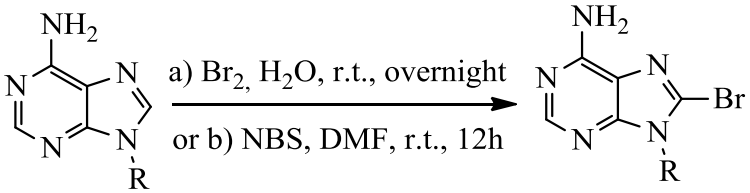


Scheme 31. Second attempted arylation of adenine (**4**).

As these approaches of arylating adenine did not give good yields, another synthetic route was designed for this target molecule (**3d**) (see Strategy 4).

3.4.4. Bromination of the C-8 Position

Bromination of the 9-alkylated purines (**7**) was carried out by two methods depending on the substrate (Scheme 32).



Scheme 32. Methods of brominating the 9-substituted adenines (**7**) that were used in this project.

For 9-cyclopentyladenine (**7b**), a literature procedure which employed a mixture of liquid bromine and water was used. This gave a good yield of 67%.[69] The same procedure was also used on 9-(cyclohexyl)methyladenine (**7c**) and resulted in a similar yield (66%). For 9-benzyladenine, the reported procedures involve the use of *N*-bromosuccinimide, in

chloroform at reflux[70] or DMF at ambient temperature.[34,71] Both these approaches were attempted and the results are presented in the table below (Table 7).

Table 7. Results of bromination of 9-benzyladenine (**7a**) with *N*-bromosuccinimide.

Entry	**NBS [eq]**	**Solvent**	**T [˚C]**	**t (lit.) [h]**	**t (exp.) [h]**	**Yield (lit.) [%]**	**Yield (exp.) [%]**
1	3	DMF	r.t.	12	24	23	42
2	5	$CHCl_3$	reflux	3	8	41	16

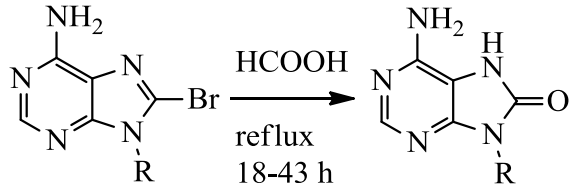
Entry 1 – reference [34].
Entry 2 – reference [69].

For both these reactions, TLC at the conclusion of the reaction time given in literature indicated incomplete conversion. Using DMF as the solvent (Entry 1), after 24 h, TLC still showed incomplete conversion but more conversion than at 12 hours. For the reaction with chloroform at reflux temperature (Entry 2), TLC indicated nearly full conversion after 8 h, although a second spot formed close to the baseline, so conversion may not have been to the desired product, but to a much more polar by-product that was not isolated.

Purification for both reactions was carried out as described in literature for each synthesis. The work-up for the reaction in DMF was flash chromatography, whereas for that in chloroform, the crude reaction mixture was washed with sodium sulfite and sodium chloride solutions. The aqueous work-up may have partially been the cause of the low yields in the second reaction (16%), and may have been unnecessary considering that a flash was sufficient to isolate the product for the first reaction (42%). In addition, the scale of the reactions (0.65 mmol – 0.86 mmol) may have been too small to be manageable and reflect losses during purification rather than indicating inefficiency in the reaction itself. It may be desirable to run the reaction using DMF as a solvent over a longer period of time if this was to be repeated.

3.4.5. Hydrolysis of 8-Bromoadenines

The three 9-substituted-8-bromoadenines (**6a**, **6b** and **6c**) obtained were hydrolysed using the same method as described in Section 3.2.4 (Scheme 33). The benzyl and cyclopentyl substrates (**3a** and **3c**) were hydrolysed overnight (20 h and 18 h, respectively). It should be mentioned that these reactions were not followed on TLC or NMR during the reaction period and these reaction times are therefore not optimised. However, based on TLC, the cyclohexylmethyl substrate (**6c**) seemed to require more time to obtain full conversion.



6 **3**

a: benzyl
b: cyclopentyl
c: (cyclohexyl)methyl

Scheme 33. Hydrolysis of 9-substituted 8-bromoadenines using formic acid.

TLC of the reaction mixture after 19 h indicated incomplete conversion. After 24 h, the conversion appeared to be greater. After 43 h, it appeared that the reaction had gone nearly to completion so it was worked up and purified. It is unknown what the cause of this much longer reaction time.

The need for the longer reaction time (43 h) is under doubt as, later in the project, mixtures of 8-bromo- and 8-chloroadenines (**6** and **8**) are hydrolysed with good yields (>90%) with a much short reaction time of only 18 h. This would indicate that hydrolysis of 8-bromoadenines are possible with a shorter reaction time than used here. It would be desirable to repeat this reaction to confirm whether or not this long reaction time is needed, but this was not prioritised as sufficient amounts of the target compound (**3c**) had been obtained and due to time considerations of the project. The results of the three hydrolysis reactions are shown in Table 8.

Table 8. Results of hydrolysing 9-substituted-8-bromoadenines.

Entry	R	t [h]	Yield [%]
1	**a:** Benzyl	20	85
2	**b:** Cyclopentyl	43	78
3	**c:** (Cyclohexyl)methyl	18	89

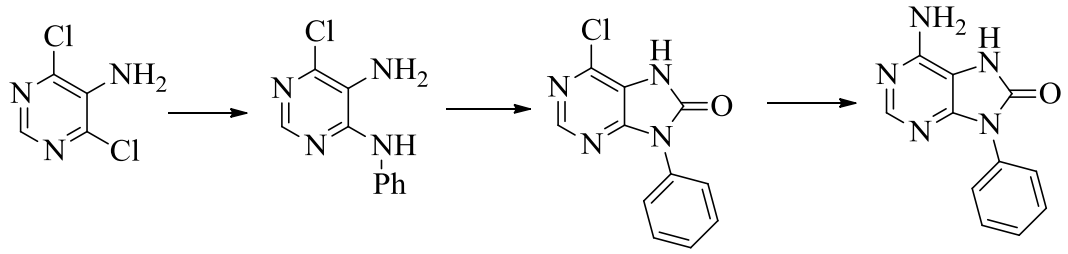
3.4.6. Conclusions

Strategy 3 was initially the least favoured of the three proposed routes in Section 1.3, since it had the worst reaction economy as the substitution on *N*-9 is carried out in the first step. However, the results obtained are the best out of the synthetic routes that had originally been envisioned, with overall yields of 19%, 37% and 42% for **3a**, **3b** and **3c**, respectively.

3.5. Strategy 4 – Synthesis of 9-Phenyl-8-oxoadenine from 4,6-Dichloro-5-aminopyrimidine

3.5.1. General

The second strategy for obtaining 9-phenyl-8-oxoadenine (**3d**) for biological testing was to aminate 4,6-dichloro-5-amino-pyrimidine (**9**) with aniline, then ring-close the substituted pyrimidine (**10a**) with carbonyldiimidazole. The final step would be to aminate at C-6 with liquid ammonia or ammonium hydroxide.

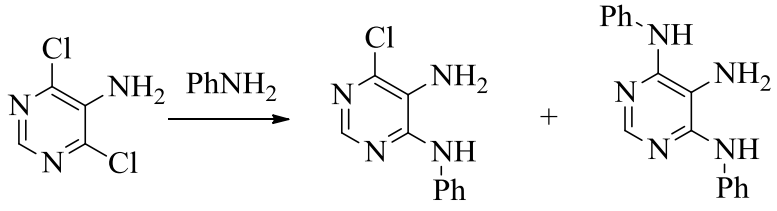


9 **10a** **11** **3d**

Scheme 34. Alternative method of synthesis of 9-phenyl-8-oxoadenine (**3d**).

3.5.2. Amination of 4,6-Chloro-5-aminopyrimidine

The amination of compound **9** was carried out following the general scheme shown below (Scheme 35) with various reaction conditions (Table 9).



9 **10a** **10b**

Scheme 35. Amination of 4,6-dichloro-5-aminopyrimidine (**9**) with aniline.

Table 9. Different conditions used to aminate 4,6-dichloro-5-aminopyrimidine (**9**) (Scheme 35).

Entry	S.M. [mmol]	Aniline [mmol]	Reaction Conditions	Yield of monoaminated (10a) [%]	Yield of diaminated (10b) [%]
1	2	2.6	6 mL water, 1 mL ethanol, 0.1 mL conc. HCl, reflux, 14 h	69	-
2	1.5	2	2.1 mmol triethylamine, 5 mL *n*-BuOH (118 ˚C), reflux, 3 d	68	-
3	2	~10	Aniline, 118 ˚C, 3 h	-	15 and 55 **10b**.**HCl**

4,6-Dichloro-5-aminopyrimidine (**9**) was first aminated with aniline to give 6-chloro-N^4-phenylpyrimidine-4,5-diamine (**10a**) following a literature procedure (see Table 9, Entry 1).[52] This reaction was also run in parallel with a longer reaction time (14 h) because TLC showed presence of starting material in the reaction mixture after the prescribed time (8 h). The longer reaction time had no affect on the conversion or yield.

For comparison, the amination was also carried out using conditions that have been used earlier in the Gundersen group at UiO for amination of **9** with a primary amine. This procedure involved refluxing the starting material with the appropriate amine in the presence of triethylamine in *n*-butanol (Entry 2).[72] Unfortunately, the method required a longer reaction time for complete conversion, had a more difficult separation at work-up, and gave no improved yield. In addition a disubstituted salt was observed in the crude reaction mixture (crude ratio: 77:23 **10a**:**10b**.**HCl**). This method was also carried out in parallel with double the amount of aniline to ascertain if the reaction time could be reduced, but this resulted only in a higher percentage conversion to the disubstituted salt (crude ratio: 23:77 **10a**:**10b**.**HCl**).

The disubstituted compound and associated salt were identified from the third reaction of the pyrimidine with pure aniline (Table 9, Entry 3), where no monosubstituted product was

observed, but there was a quick conversion to the disubstituted product **10b**. In this method, the starting material was simply heated in aniline at 118 ˚C (the boiling point of *n*-butanol). The method was carried out for comparison purposes with the other two methods, but also to obtain the expected diaminated compound that should be produced with a large excess of aniline for use in identification in the other reactions (Entry 3).

The compound **10b** was obtained mostly as a hydrochloride salt that could be converted to **10b** by treatment with a base. In hindsight, it would have been logical to treat the crude mixture with a base before work-up to convert the hydrochloride salt to compound **10b**. However, the information which was required from this experiment was obtained so no further work was carried out.

There were reactions carried out using the Gundersen group's conditions with different amounts of aniline (Table 10, Entries 1 and 2). These reactions were followed by examination of the crude by NMR spectroscopy after 1, 2 and 3 days and the results are presented in the table below (Table 10). The results indicate that complete conversion of the starting material requires three days, furthermore, allowing the reaction to run this long results in the production of a significant amount of dialkylated product. Based on ratios, using 1.2 eq of aniline and a reaction time of 3 days should result in the highest yield of compound **10a**. However, if separation of 9 and 10a is simpler than that of 10a and 10b.HCl, using 2.5 eq of aniline and a shorter reaction time may be preferable.

Table 10. Ratios of the starting material to monoalkylated and dialkylated salt (**9**:**10a**:**10b.HCl**) in the reaction mixture after 1, 2 and 3 days and products from crude NMR spectroscopy.

Entry	**Aniline**	**1 day [%]**	**2 days [%]**	**3 days [%]**	**Yield (10a) [%]**
1	1.2 eq	60:40:0	41:59:0	0:77:23	68
2	2.5 eq	38:62:0	0.1:44:56	0:23:77	~16, impure

For the amination step, it would seem that the first method was the most preferable method. However, it is curious to note that the reaction occurs much faster under acidic conditions

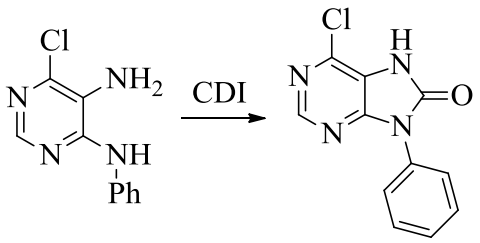
(Table 9, Entry 1) than basic conditions (Entry 2), with both methods giving similar yields. It should be noted that the first method (Entry 1) involved direct recrystallization of the crude mixture without the opportunity to examine the products at this stage by NMR spectroscopy. It is possible that the disubstituted product was produced in the reaction, but this was not ascertainable after following the given procedure and work-up. For all other reactions, flash chromatography was used for purification.

One possible reason for the faster reaction rate under acidic conditions may be that the acid catalyses the reaction by protonating on one of the ring-nitrogens,[28] thus reducing the electron-density in the ring further and increasing the attractiveness of C-4 and C-6 to nucleophilic attack. The ability of acid to accelerate nucleophilic attack on heterocyclic compounds has been discussed before in literature.[73,74] This has perhaps a parallel in the acid-catalysed hydrolysis of 8-halopurines to 8-oxopurines (see Section 2.6).

The purpose of the base in the base-catalysed reaction seems to be to remove the excess hydrogen chloride which is the by-product of the aromatic substitution reaction. With a more reactive nucleophile such as a benzyl amine, the removal of the acid, which could catalyse the reaction, seems to affect the rate of the reaction less than with a less reactive nucleophile such as aniline. It is in any case not surprising that, under similar conditions, the reaction with aniline is slower than that with the benzyl amine when the difference in nucleophilicity between these types of amines is taken into consideration – i.e. benzyl amine being more nucleophilic than aniline.[75]

3.5.3. Cyclisation of a Diamino-pyrimidine with Carbonyl Diimidazole

The cyclisation step was carried out on compound **10a** with carbonyldiimidazole (CDI) (Scheme 36). This reaction had been reported to be employed on similar substrates (2-phenyl-4,5-diaminopyrimidine and 4,5-diamino-6-chloropyrimidine) with good results (82% and 86%, respectively).[76,77] The first method had used THF as the solvent at ambient temperature for 2 h while the second employed 1,4-dioxane at reflux for 24 h.



10a **11**

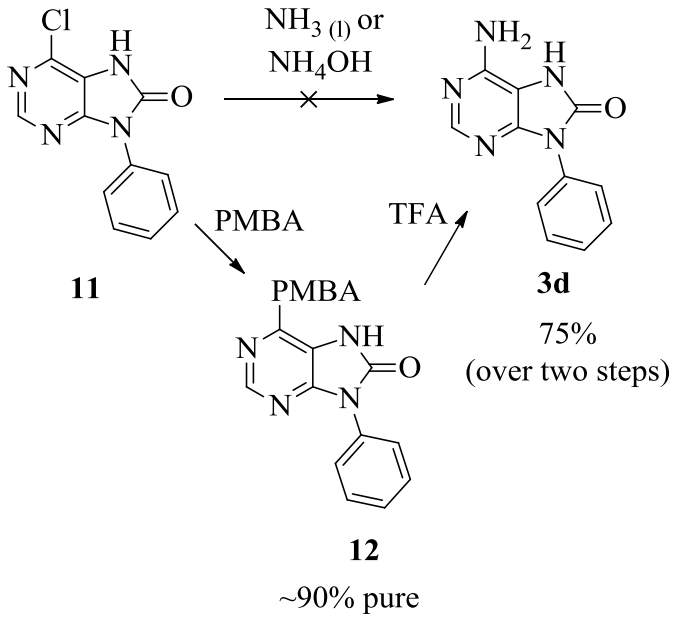
97%

Scheme 36. Cyclisation of compound **10a** using carbonyldiimidazole.

It was decided to use the less harsh conditions as a starting point, and after 6 h stirring in THF at ambient temperature, the reaction had gone to completion. Only the product and imidazole were observed in ^{1}H NMR spectrum of the crude mixture. After flash chromatography, the product was obtained in good yields (97%).

3.5.4. Amination of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one

The final step of substituting the chlorine atom on C-6 with an amino group was more difficult than expected (Scheme 37). Direct substitution of the chloro-substituent with an amino group has not been successfully carried out previously on 8-oxoadenines. The closest related reactions are amination on halopurines with primary and secondary amines on 8-oxo-halopurines.[78-80]



Scheme 37. Synthesis route of the target molecule (**3d**) from compound **11**.

Treatment of compound **11** with liquid ammonia at ambient temperature gave no reaction after 24 h. Due to restrictions on the sealed vessels available; this was not attempted at higher temperatures. Reaction with concentrated ammonia solution (28%) gave no reaction at ambient temperature. At 100 ˚C after 18 h, a small but visible amount of product was identified *via* inspecting the ^{1}H NMR spectra of the crude mixture.

The temperature was raised to 120 ˚C and the rate of the reaction increased slightly with approximately ~9% per day. When the temperature was raised to 150 ˚C, the reaction vessel did not tolerate these high pressures and the lid exploded after 2.5 h at this temperature. The amount of conversion after this time was estimated to be 10% over those 2.5 h from the ^{1}H NMR spectrum of the residue in the reaction vessel. The reaction was attempted once more at 130 ˚C and after being allowed to stir at that temperature for 7 days, the result was only slightly more than 50% conversion.

Compound **11** proved surprisingly resistant to amination attempts. One explanation may be due the presence of the acidic proton on *N*-7 which would possibly deprotonate in a basic environment and make the purine ring less electrophilic – i.e. more electron-rich. The amination reaction installing only an amino group has in any case not previously or presently been reported on an 8-oxoadenine.

It was decided to treat **11** with a more nucleophilic primary amine and we chose to use a benzylamine and cleave off the attached benzyl group in the final step with acid.[81] Benzylamines are more nucleophilic than ammonia[75] and in particular *para*-methoxybenzylamine, with its electron-donating methoxy group is even more nucleophilic and should have a greater rate of reaction in a nucleophilic attack than both unsubstituted benzylamine and ammonia.[82]

The amination was thus carried out with *para*-methoxybenzylamine. The first experiment was carried out in ethanol at reflux. However the conversion was low (~50%) and the solvent was changed to *n*-butanol, providing a much higher reflux temperature and, as a result, full conversion. After partial purification, the product (**12**) was obtained ~90% pure and after cleavage of the benzyl group and purification *via* flash chromatography, this impurity was removed to give the target molecule (**3d**) pure (yield over two steps = 75%).

A small amount of the impurity (20 mg) was isolated during flash chromatography in the final step and purified using recrystallization with chloroform and hexanes for structural determination. Structure elucidation with NMR spectroscopy and MS indicated that compound **12** had been isolated. However, when the amination step was repeated and the product recrystallized from the mixture, the two spectra were slightly different. The difference was mainly in the benzylmethylene protons. These appeared as a singlet in the impurity and a doublet in what was presumably compound **12**.

It is thus proposed that this impurity is in fact the hydrochloride salt of **12**, which is logical since there was no base present in the reaction to consume the hydrogen chloride produced. Nether was neutralisation carried out during the work-up. If this was to be repeated, we would suggest the use of a base (perhaps triethylamine) that would both consume the hydrogen chloride in step 1. In fact, most of the previous work in this area (excluding where microwave irradiation has been used), has included some triethylamine in the reaction mixture.

3.5.5. Conclusions

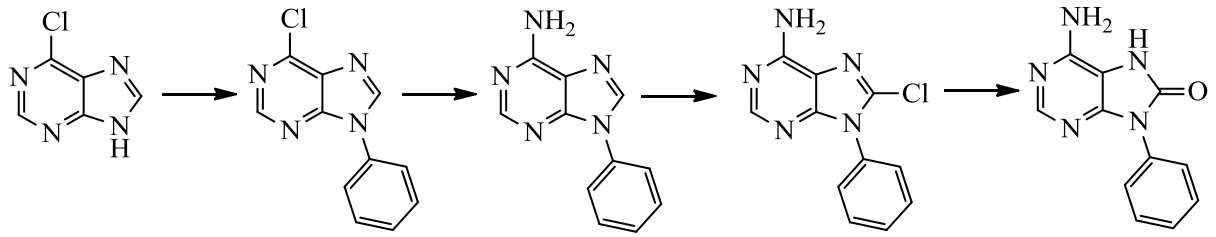
This synthetic strategy is clean, efficient and has given the target molecule (**3d**) in good yields (50% over 4 steps). It is undoubtedly an acceptable method to obtain 9-phenyl-8-oxoadenine. It would be interesting to carry out this synthesis route on the other target molecules to compare results.

3.6. Strategy 5 – Alternative Synthesis of 9-Phenyl-8-oxoadenine

3.6.1. General

Even though sufficient amounts of the target compound (**3d**) had been obtained *via* Strategy 4 (see Section 3.5), it was desirable to establish if a synthetic route more similar to that used successfully for benzyl, cyclopentyl and cyclohexylmethyl analogues could be followed to obtain compound **3d** – i.e. 9-substitution, halogenation then hydrolysis. In addition, the route envisioned would allow for work in the area of the lithiation project already in progress in the Gundersen group at UiO.

The synthetic route presented here (Scheme 38) was designed with the intention of acquiring a route that mirrored the most successful route used for the other target molecules – alkylation of adenine, followed by halogenation and hydrolysis. As indicated earlier in Section 3.4.3, attempts to carry out direct arylation of adenine were met with limited success. It was decided to begin with 6-chloropurine (**13**) and arylate according to a procedure used previously in the Gundersen group at UiO.[45] This would be followed by amination, halogenation and hydrolysis (Scheme 38).

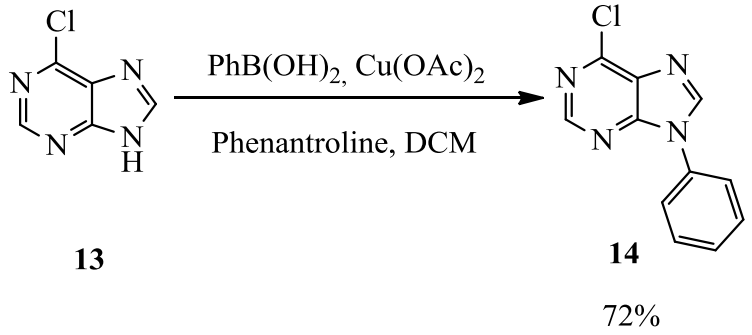


13 **14** **7d** **8d** **3d**

Scheme 38. Third proposed synthesis for 9-phenyl-8-oxoadenine (**3d**).

3.6.2. Arylation of 6-Chloropurine

The arylation of 6-chloropurine (**13**) was carried out under similar conditions to the reactions shown in Section 3.4.3, but using anhydrous copper(II) acetate instead of the monohydrate (Scheme 39), resulting in 72% yield of 6-chloro-9-phenylpurine (**14**).



13 **14**

72%

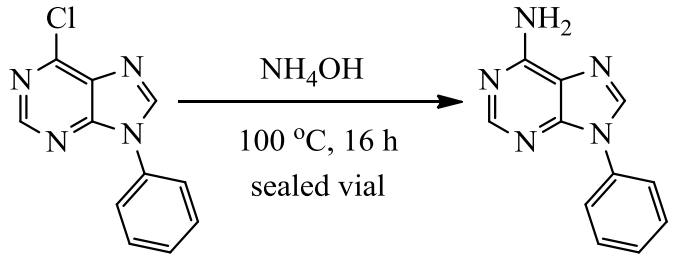
Scheme 39. Copper-catalysed coupling of 6-chloropurine (**13**) and phenylboronic acid.

The comparably good yields obtained here may indicate that the results of the copper-catalysed coupling may depend on the substrate used. It has been reported that copper(II) can form different complexes with adenine (**4**) and other purine-derivatives.[83] Thus, in the case of arylation of adenine (**4**), it may be that either the product (**7d**) or the starting material (**4**), or both, are forming complexes with the copper that are not able to be isolated using the types of purification employed – flash chromatography, with or without aqueous work-up.

The arylation of 6-chloropurine (**13**) showed better regioselectivity than the arylation of adenine (**4)** under similar conditions. Only the 9-substituted product (**14**) was produced with 6-chloropurine while the 9-, 7- and 3- substituted products (**7d**, **29d** and **28d**) were all observed from arylation of adenine. The ability of purines to form complexes with copper may also affect the regioselectivity of the reactions. For example, under the conditions used, copper could prefer to form a complex at *N*-3 and *N*-9 with 6-chloropurine (**13**) but with N^6 and *N*-7 with adenine (**4**). In any case, it was clear that the method giving the highest yields of 9-arylated purine in our hands was to arylate 6-chloropurine (**13**).

3.6.3. Amination of 6-Chloro-9-Phenylpurine

The amination of compound **14** was carried out under the same conditions as those that were employed on the 8-oxo-analogue (**11**) (Scheme 40). Compound **14** was heated in concentrated ammonium hydroxide at high temperature in a sealed vial overnight. This gave an excellent yield of 95%. The combined yield for these two steps (68%) was thus much higher and comfortable to work with than the 20% obtained in the course of Strategy 3 (Section 3.4.3).



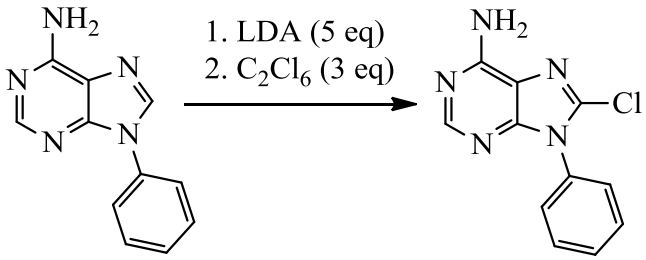
14 **7d**

95%

Scheme 40. S_NAr of chlorine in compound **14** with an amino group.

3.6.4. Halogenation of 9-Phenyladenine

The method of halogenation chosen was deprotonation of 9-phenyladenine (**7d**) using lithium diisopropylamide (LDA) generated *in situ*, followed by capture of the adenine anion with an appropriate source of electrophilic halogen (Scheme 41). There are no reported methods for carrying out this transformation and use of the lithiation/halogenation method added to an ongoing project in the Gundersen group at UiO which has the goal of screening substrates to ascertain which are amenable to undergoing halogenation at the C-8 position *via* this method (see Chapter 4).

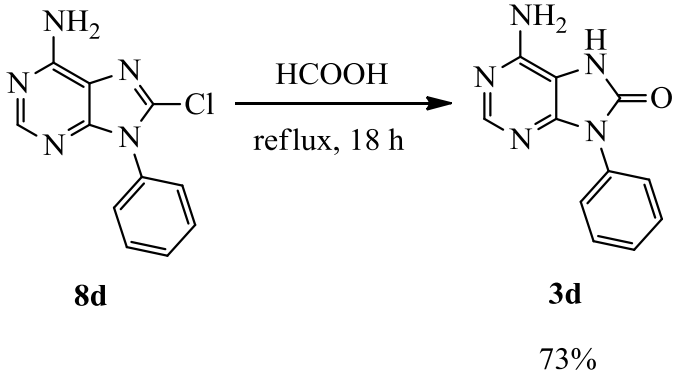


Scheme 41. Lithiation of **7d** and capture with hexachloroethane.

For reasons that will be discussed in Chapter 4 (dedicated to the lithiation/halogenation research that was undertaken as part of a larger group project), the chosen electrophilic source was hexachloroethane. The reaction gave a yield of 67% of the desired product (**8d**) after aqueous work-up and flash. This result was as good as the highest yields seen when we brominated with bromine and water. Still, it would have been interesting to carry out a bromination using liquid bromine and water on this substrate (**7d**) in order to compare the results.

3.6.5. Hydrolysis of 8-Chloro-9-phenyladenine

Compound **8d** was hydrolysed to the target molecule **3d** using formic acid as described earlier in Section 3.2.4, giving a 73% yield (Scheme 42).



Scheme 42. Hydrolysis of 8-chloro-9-phenyladenine (**8d**) to the target molecule (**3d**).

3.6.6. Conclusions

This synthetic route gave an overall yield of 33% over 4 steps. The number of steps is the greatest difference compared to the similar routes where adenine is alkylated directly. On the other hand, this route (like that being with 4,6-dichloro-5-aminopyrimine in Strategy 4) has the advantage of being regioselective in the first step where the side-group is established. None of the other alkylation or arylation experiments carried out in the course of the current project are close to being regioselective – all resulted in reaction mixtures with more demanding purification. It is clear from both Strategy 4 and Strategy 5 that the exploitation of regioselective reactions is desirable to allow for more straightforward purification and conservation of time and resources.

CHAPTER 4

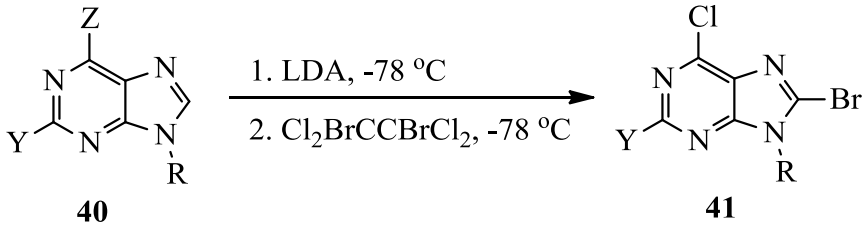
4. LITHIATION AND HALOGENATION EXPERIMENTS

4.1. Background to the "Lithiation Project"

There are several reasons why a method for fast and efficient halogenation on a variety of substituted purine compounds is interesting. As mentioned earlier, 2-, 6- and 8-halopurines are important intermediates for the functionalization of purines, which are interesting and potential biologically-active compounds. There is thus a need to survey the flexibility of our method for halogenating the C-8 position of substituted purines, with the intention of creating more functionalisable molecules.[51]

The "lithiation project" is an ongoing project in the Gundersen group at UiO that has been worked on by several other post-graduate students.[84,85] This ongoing project has focused on the possibilities of efficiently generating 8-halopurines by lithiation (using LDA) and subsequent capture with a halogen electrophile (e.g. hexachloroethane, tosylchloride, dibromotetrachloroethane, and cyanogen bromide or cyanogen iodide).

Several types of compounds with a variety of substitution patterns have already been surveyed using this method. Most of the recent work done on this project has been carried out on 6-chloro-9-benzylated compounds.[49,85] In the past year, studies have been conducted on a range of 6-chloro- and 6-amino-9-alkylated purines (Scheme 43).[84]



40 **41**

Y = H, Cl, NO_2
Z = Cl, NH_2,
R = benzyllic groups, tetrahydro-2H-pyran-2-yl, Me, Et, Allyl

Scheme 43. Work performed previously on this project.[49,84]

The experiments described in this chapter provide an alternative method of introducing a halogen on the C-8 of a 9-substituted adenine that can later be hydrolysed to the target molecules, and as such can be considered a direct parallel to the bromination experiments carried out in Section 3.4.4. The results will also add to the library of results obtained so far by the other members of the Gundersen group at UiO.

4.2. Substrates used in this Study

The substrates used in this part of the study are shown in Figure 20 below. A variety of aromatic and cyclic ring systems are represented. Most are adenine-derivatives and one is a 6-chloropurine derivative. All the compounds are intermediates in strategies to obtain the target molecules and have all been synthesised from adenine (**4**) or 6-chloropurine (**13**) as described in Chapter 3.

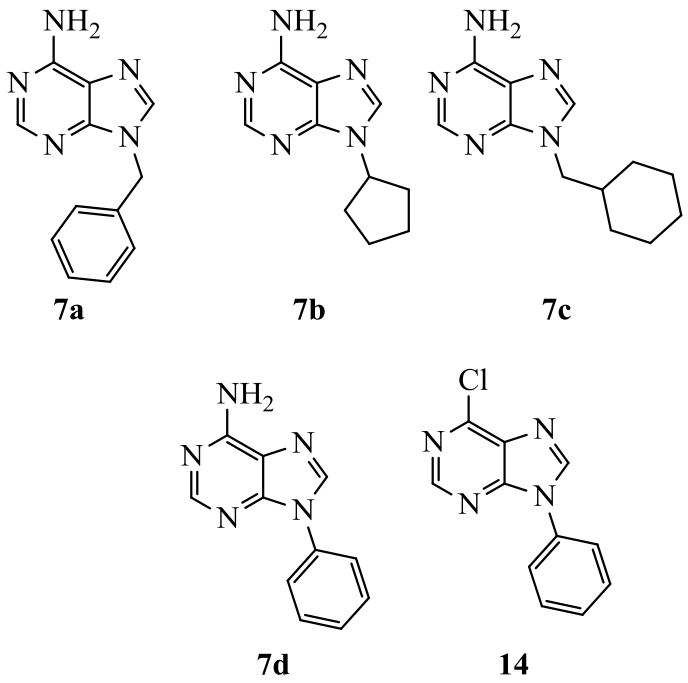
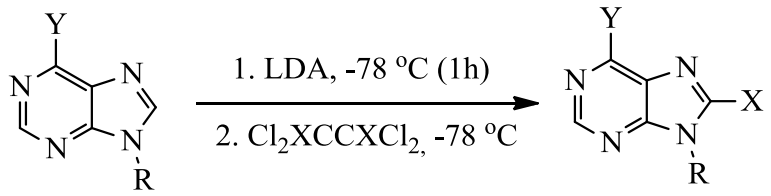


Figure 20. Substrates upon which lithiation / halogenation was attempted.

4.3. General Procedure

The common method used for the lithiation and halogenation of 6,9-substituted purines is shown below (Scheme 44).



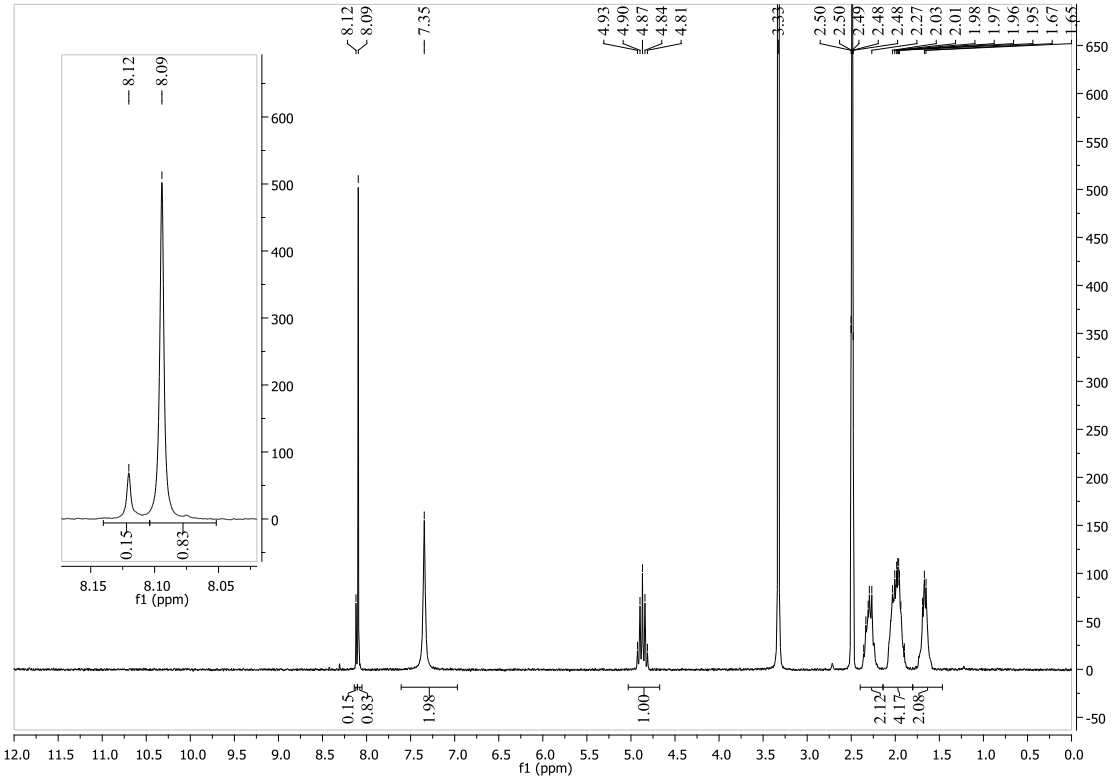
7a: Y = NH_2, R = benzyl
7b: Y = NH_2, R= cyclopentyl
7c: Y = NH_2, R= (cyclohexyl)methyl
7d: Y = NH_2, R = phenyl
14: Y = Cl, R = phenyl

Scheme 44. The common method for halogenation of the relevant purines is shown above.

4.4. Halogenation of Adenine Substrates

4.4.1. Capture with 1,1,2,2-Tetrachloro-1,2-dibromoethane

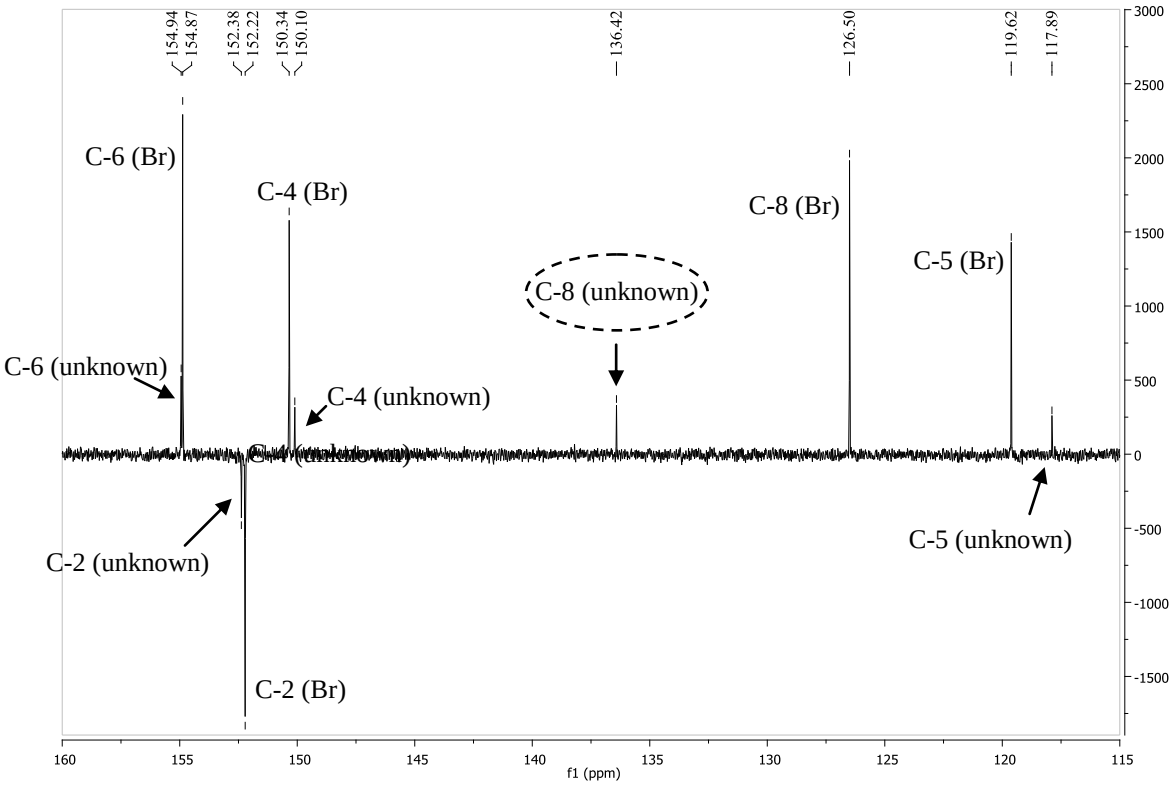
Lithiation and capture with tetrachlorodibromoethane was carried out on compounds **7b** and **7c** using 5 eq of LDA and 3 eq of the brominating agent. This gave a product which appeared nearly pure and was in agreement with the data obtained earlier. However, a small impurity was visible on the ^{1}H NMR spectra at 8.12 ppm and 8.14 ppm for compounds **7b** and **7c**, respectively (Spectrum 4). This peak was in the typical purine region, but there did not appear to be any other unexpected peaks visible in the spectra.



Spectrum 4. ^{1}H spectra of the isolated product from the lithiation and bromination of compound **7b**.

The impurity was not removed or reduced by flash chromatography or recrystallization in several combinations of solvents. The fact that it had very similar properties to the expected product led to the hypothesis that the impurity was actually another purine compound with slightly different substituents.

From the carbon NMR spectrum of the product mixture (Spectrum 5), it became apparent that the impurity had a full set of purine signals which mirrored the shifts observed for the brominated compound, with the exception of C-8 (circled in Spectrum 5). It was then hypothesised that it was the identity of the substituent on the C-8 that was different in the by-product. In addition, the substituent would have to be more strongly electron-withdrawing, thereby deshielding its neighbouring carbon more than bromine.



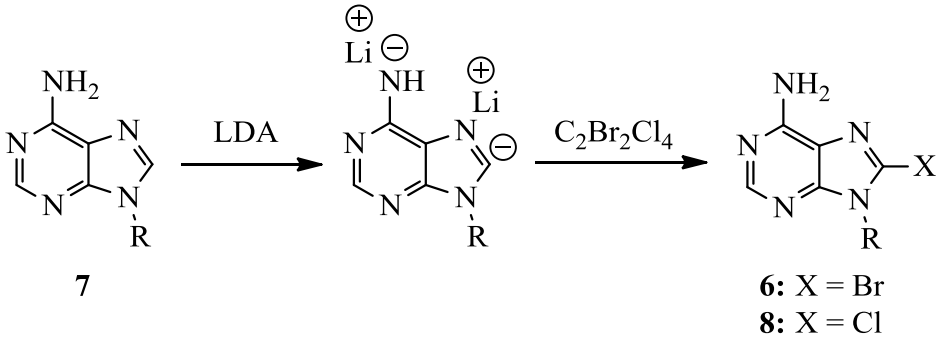
Spectrum 5. Purine region of the ^{13}C spectra of the isolated product from the lithiation and bromination of compound **7b**.

The logical conclusion was that a small percentage of the starting materials were being chlorinated instead of brominated, resulting in the corresponding 9-substituted 8-chloroadenines (**8b** and **8c**). The identity of the by-product was confirmed by both ESI-MS and subsequently, the synthesis of the 8-chloroadenine analogues and comparison of their NMR data.

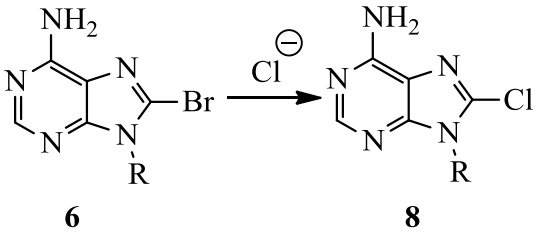
The undesired chlorination has not been observed previously in this project, although another Masters student in the Gundersen group at UiO subsequently discovered a similar problem as a side-product to bromination of purine-derivatives using this method.[84]

The suggested mechanism for capture of the anion intermediate with an electrophilic halogen (Scheme 45) has been shown in Section 2.3.3 (see Scheme 11).[85] Bromination should be the dominant transformation, as the chlorine atoms should have greater electron-drawing strength, making the bromine atoms more electrophilic than any of the chlorine atoms. This hypothesis is consistent with the observation that the major product is the brominated product.

There are two theories proposed as to why this occurred and these are set out in Scheme 45 and Scheme 46.



Scheme 45. The proposed synthetic route used to create the mixtures of brominated (**6**) and chlorinated (**8**) products.



Scheme 46. Possible nucleophilic aromatic substitution of 8-bromo-9-substituted adenines (**6**) by free chlorine anions.

In the first proposed mechanism (Scheme 45), the purine nucleophile could attack a chlorine atom in the instead of a bromine atom, eliminating either a bromine or chlorine atom in the process. This could be caused by the presence of a bulky substituent on the 9-position, making the smaller chlorine atom more sterically favourable for this substrate. This yields the chlorinated product (**8**) directly.

The second suggested mechanism is that the attack on the bromine atom could occur while the haloethane is in a less favourable Gauche conformation and result in the elimination of a chlorine atom. This free chlorine nucleophile is free to then attack the brominated compound and carry out nucleophilic aromatic substitution, also giving the chlorinated product (Scheme 46).

It is worth noting that bromination using the lithiation capture-method has been carried out in the Gundersen group at UiO on the less sterically-hindered 9-methyl- and 9-ethyladenines.[84] In the first case, no chlorination was observed, but for the ethyl substrate, 8-10% of the chlorinated product was reported. It may be that sterical factors play a part in this mechanism – pointing to the situation in Scheme 45 as being more likely.

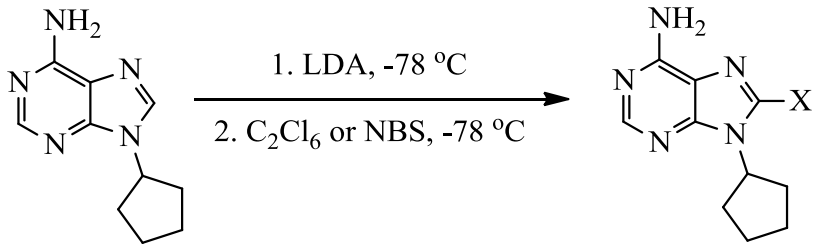
Since no mechanistic studies have been carried out on this reaction, no conclusions will be drawn in this work. However, it is a consideration to be aware of, if one uses dibromotetrachloroethane as a brominating agent. In our project, the presence of the chlorinated product (**8b** and **8c**) did not affect the outcome, since both the chlorine and bromine act as good leaving groups in the hydrolysis step. The approximate isolated yields of the bromination reactions (calculated from NMR) are given in Table 11.

Table 11. Results of bromination using tetrachlorodibromoethane.

Entry	**R**	**Ratio 6:8**	**6:8 [%]**	**Total yield [%]**
1	**b:** Cyclopentyl	10:2	62:12	~74
2	**c:** Cyclohexylmethyl	10:4	57:23	~66

4.4.2. Attempted Optimisation of the Reaction Conditions using 9-Cyclopentyladenine

To simplify matters, it was decided to try different electrophilic species that could only donate one species of halogen atom. In addition, since conversion was less than 100% in the original reactions, a few different sets of conditions were tried out. The chosen substrate was the cyclopentyl analogue and the results are set out in Table 12.



7b

6b: X = Br
8b: X = Cl

Scheme 47. General reaction for lithiation/halogentaion of 9-cyclopentyladenine (**7b**).

Table 12. Conditions used for optimisation of the reaction shown in Scheme 47.

Entry	**Electrophile source**	**"E^+"**	**LDA [eq]**	**t (step 1) [h]**	**t (step 2) [h]**	**Approximate conversion (NMR) [%]**	**Yield [%]**
1	$BrCl_2CCCl_2Br$	Br/Cl	2.8	1	4	60	~53
2	$BrCl_2CCCl_2Br$	Br /Cl	5	1	4	75	~74
3	NBS	Br	5	1	4	80	65
4	NBS	Br	5	2	4	80	57
5	NBS	Br	5	1	20 (to r.t.)	75	60
6	C_2Cl_6	Cl	5	1	4	87	69
7	C_2Cl_6	Cl	10	1	4	78	86

From Entries 2 to 6 in Table 12, it can be observed that the approximate conversions and yields remain around the same area (70-80% and 60-70%, respectively). In the end, it was decided to use 5 eq LDA and hexachloroethane as the electrophile donor. The reasons for this were several.

Firstly, the by-product of using NBS, succinimide, proved more difficult to remove than the by-product of the other two reagents, tetrachloroethene. It eluted in flash chromatography

with similar retention as both the product and starting material, and required an additional aqueous work-up.

In comparison, tetrachloroethene has a boiling point of 121 ˚C and is extremely non-polar. This means that it is easy to separate from both the product and starting material if it is not already removed during evaporation of the solvent and concentration of the reaction mixture.

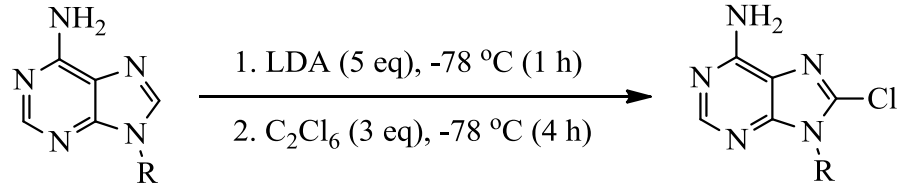
Secondly, it had been found previously that neither a longer lithiation nor reaction time appeared to affect the outcome, nor did allowing the reaction temperature to increase to ambient temperature overnight.

Finally, the amount LDA used was retained at 5 eq, despite Entry 7 perhaps indicating that yields could be improved. Entry 7 is slightly suspicious as the yield obtained was higher than the conversion indicated by crude NMR spectroscopy. The source of this discrepancy is unknown but in hindsight it would have been desirable to repeat this experiment to ascertain if this yield is reproducible. The NMR spectrum itself was not a very good one, and the conversion indicated may have been misleading.

This decision was, in the end, made for two reasons: the conversion indicated on NMR had not improved significantly and it seemed preferable to use the group's standard amount of LDA for adenine, which is 5 eq. This would allow results obtained in this project to be comparable. This decision in retrospect may be deserving of criticism.

4.4.3. Lithiation and Capture with Hexachloroethane

The final scheme for the adenine-derivatives (**7**) looked therefore as shown in Scheme 48.



7

8

a: R = benzyl
b: R= cyclopentyl
c: R= (cyclohexyl)methyl
d: R = phenyl

Scheme 48. Final standard conditions for lithiation and halogenation used in this thesis.

The standardised method was then carried out on all adenine substrates and after aqueous work-up were further purified with a flash system that seemed to give a good balance of solubility and separation. The halogenated products were obtained in moderate yields and consistent yields. The results are presented in Table 13.

Table 13. Results for chlorination *via* lithiation for the different 9-substituted adenine substrates (**7**).

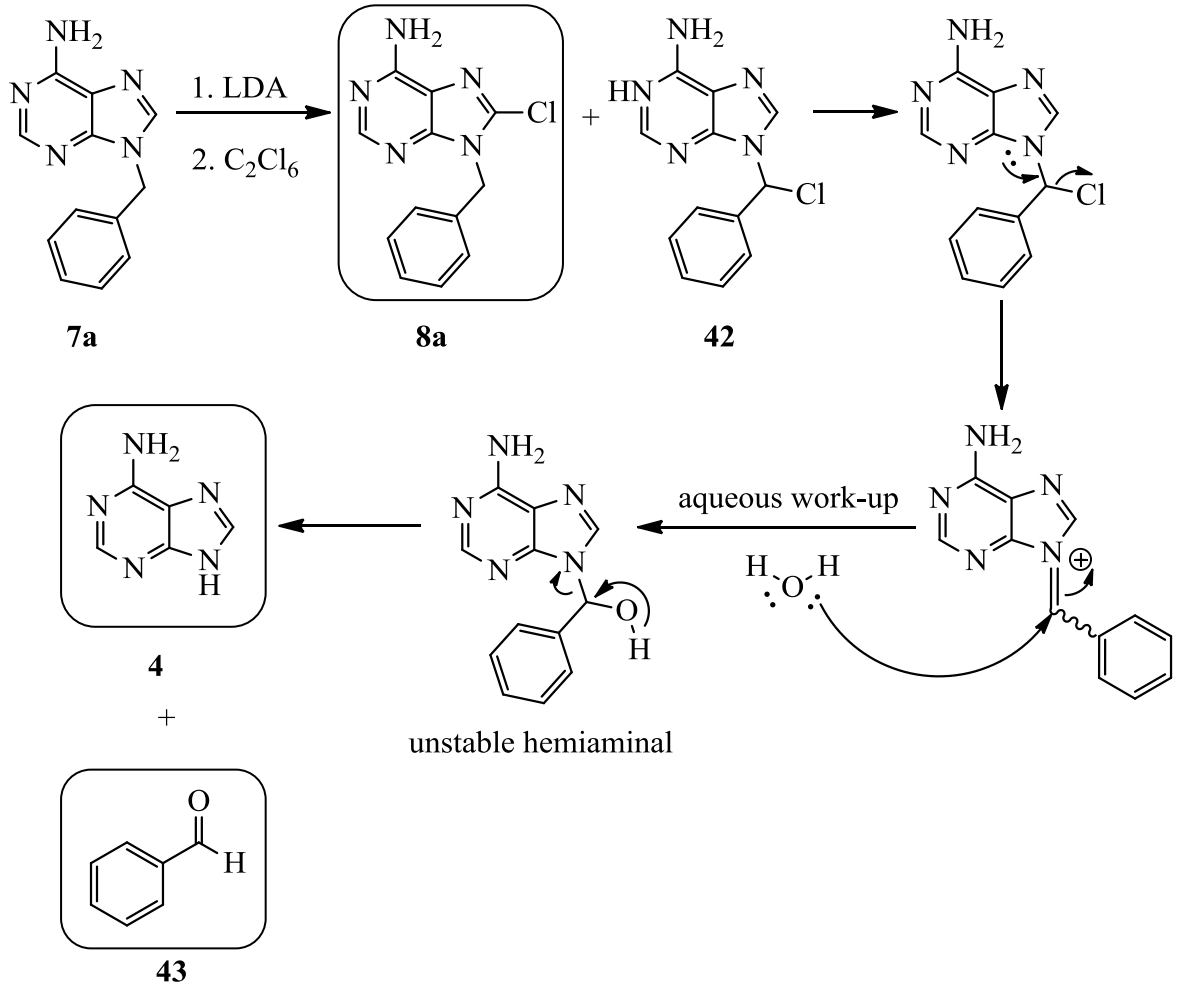
Entry	R	Yield (halogenation) [%]
1	**a:** Benzyl	Debenzylation
2	**b:** Cyclopentyl	69
3	**c:** (Cyclohexyl)methyl	68
4	**d:** Phenyl	67

One interesting result is that seen for attempting lithiation and chlorination with 9-benzyladenine (Table 13, Entry 1). Instead of a clean reaction, as seen for the other substrates, the products isolated here included (but were not limited to) starting material (~14%), product (<10%), adenine (~12%) and benzaldehyde (trace), all impure.

Debenzylation of nitrogens has been widely reported – not all methods are described here. One route is to carry out acid hydrolysis with trifluoroacetic acid – used in fact in this project to cleave a 4-methoxybenzyl group (see Section 3.4.4).[81] Hydrogenolysis with palladium or carbon is another known method.[86] It has been also been reported that a strong base and oxygen can be employed to carry out debenzylation.[86-89]

An earlier Masters student in the Gundersen group at UiO, Thywill Gamadeku, has suggested two mechanisms as the explanation for observations of debenzylation when lithiating and brominating 9-benzylated purines.[85] The first is taken from an article by Suzuki – where the deprotonation occurs on the benzylic methylene which then undergoes α-elimination and oxidative debenzylation to give the debenzylated product (after aqueous work-up) and a carbene. The carbene is then attacked by the original anion and decomposes to give trans-stilbene. The second suggestion made by Gamadeku was that it is the presence of the halogen that causes the debenzylation process to occur.

In the course of the current project, it was decided to carry out two experiments to investigate possible causes of the debenzylation. The first was to quench the reaction after the lithiation step with saturated ammonium chloride solution – this returned to quantitative amount of starting material and no indications of debenzylation. The second experiment was a deuteration experiment also carried out after the lithiation step. This experiment indicated that both the H-8 and one of the N-CH_2 protons are acidic enough to be deprotonated in the first step (with the H-8 being deprotonated to a greater extent). This indicated that it was not merely the presence of the base that caused debenzylation in the current experiment, but the addition of the halogenating agent (as suggested by Gamadeku).[85] It is proposed that a mechanism like the one presented in Scheme 49 may explain the debenzylation as a result of halogenation on the benzylic position.

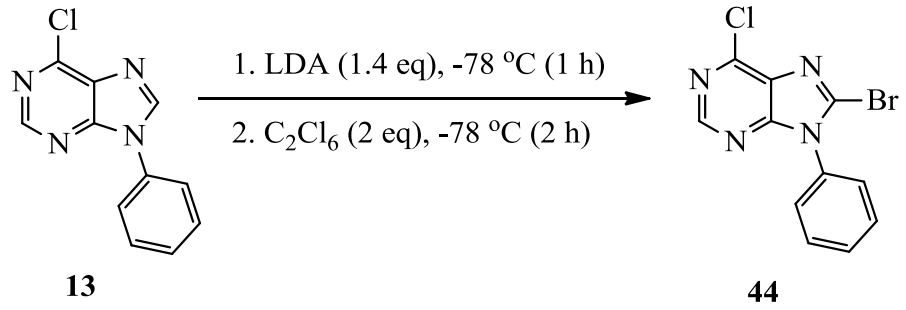


Scheme 49. Proposed mechanism for lithiation, chlorination and debenzylation.

The experiment provides a warning that carrying out deprotonation with a strong base like LDA on compounds containing side-chains with relatively acidic protons can provide the opportunity for unexpected and possibly unstable side-products. In cases where the benzylic methylene protons are the only acidic protons, the halogenation of this position may provide an efficient method to debenzylate. This remains to be seen and unfortunately fell outside the scope of this project.

4.5. Halogenation of 9-Substituted 6-Chloropurine

The lithiation and halogenation procedure was also carried out on 6-chloro-9-phenylpurine (**14**), but with fewer equivalents of LDA (1.4 eq), less brominating compound (2 eq) and shorter reaction time (2 h) (Scheme 50). This gave a pleasing 100% conversion on crude NMR, with ~5% by-product.

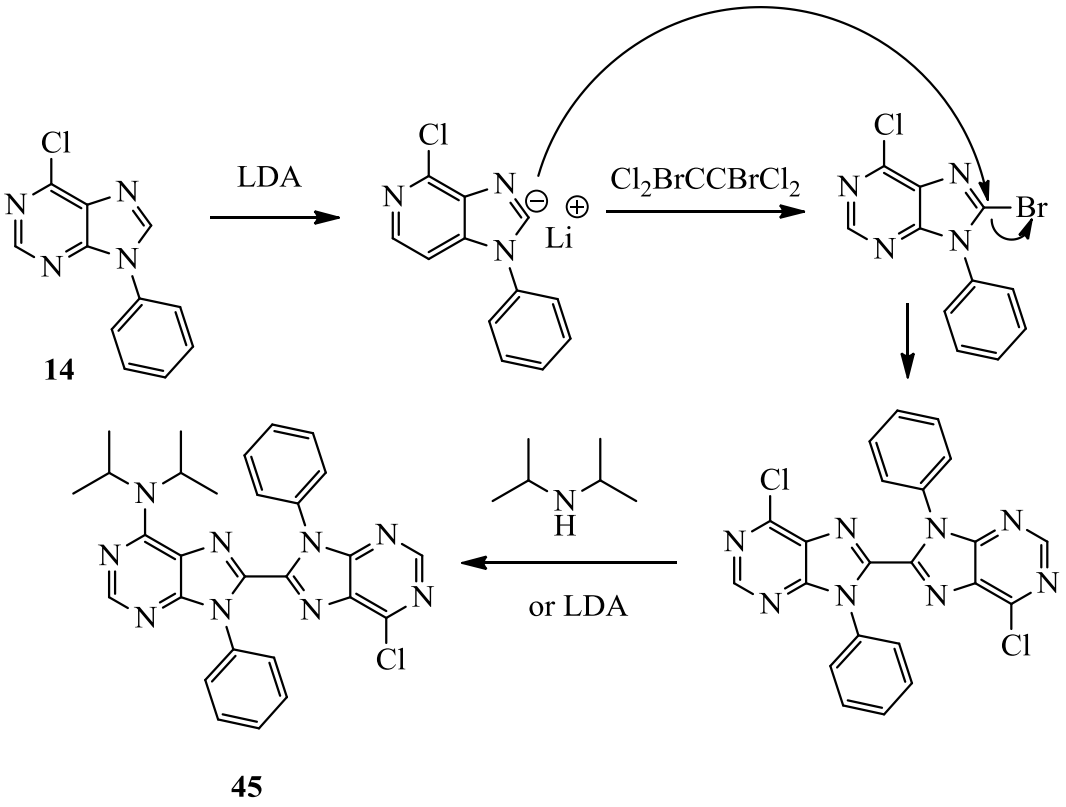


13 **44**

Scheme 50. Lithiation and halogenation of 6-chloro-9-phenylpurine (**14**).

However, upon flashing the crude mixture, it was discovered that the desired product did not tolerate silica. When the crude mixture was evaporated onto silica, the yield was only 46%. When applied as a suspension to the column, it increased to 61%. Using an aluminium oxide column had the effect of making the separation of the compounds worse due to poor solubility of the compounds at the lower polarity eluents. Recrystallization of the crude was also attempted with some limited success, reducing the amount of by-product was reduced from 5% to 1%.

If this compound was to be used as an intermediate for further reactions, it could be advisable to carry out further transformations on the crude product before purification. The by-product was obtained only as an impure mixture. Mass spectroscopy and NMR studies indicated that what was produced was a dimer with an *iso*-propylamino group attached. Below is shown a suggested structure and mechanism for the creation of a possible by-product fitting these criteria (Scheme 51).



Scheme 51. Possible by-product of lithiating and brominating 6-chloro-9-phenylpurine (**14**).

Of course, this route has no place in the synthesis of 9-phenyl-8-oxoadenine as the method used for the next amination step (heating in ammonium hydroxide) would be expected to be unselective between the 6- and 8-positions. This experiment was run anyway to see if there was a difference and the result was two monoaminated products in a 1:1 ratio and ~90% yield (Figure 21). Full characterisation was attempted on this mixture, but overlapping signals did not allow for identification with complete certainty.

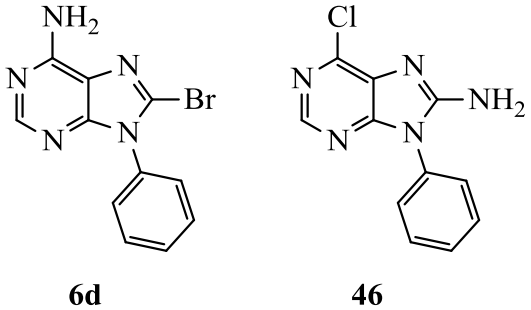


Figure 21. The two products from amination of 8-bromo-6-chloro-9-phenylpurine (**44**).

Perhaps this compound could be used as a precursor for a reaction where the identity of the halogen is important – for example, a coupling reaction. Finally it should be noted that no chlorinated compound was obtained in this experiment. The reason for this is unknown but is perhaps related to the amount of LDA required for deprotonating the adenine substrates.

4.5.1. Hydrolysis of 8-Halo-9-substituted Adenines

The 8-chloro-9-substituted adenines (**8**) obtained were hydrolysed following the same procedure as used for the hydrolysis of the 8-bromo-9-substituted adenines (**6**) (see Sections 3.24 and 3.4.5). The mixture of compounds 6b and 8b and that of 6c and 8c were also hydrolysed. The results of all the hydrolysis reactions are summarised in Table 14.

Table 14. Results of hydrolysis of compounds **6** and **8**.

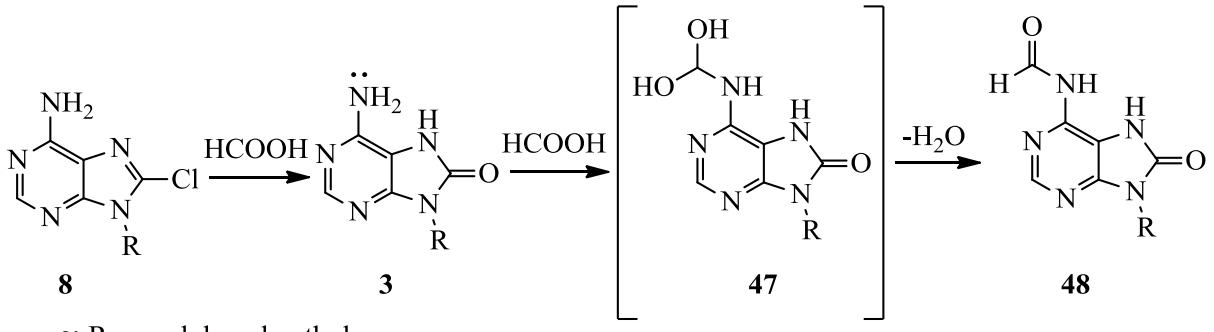
Entry	**R**	**Yield (Mixture of 8-Br and 8-Cl) [%]**	**Yield (8-Cl) [%]**	**Yield (8-Br) [%]**
1	**a:** Benzyl	N/A	N/A	85
2	**b:** Cyclopentyl	~92% (yield over two steps = 72%)	92	89
3	**c: (**Cyclohexyl)methyl	~94% (yield over two steps = 65%)	90	78
4	**d:** Phenyl	N/A	73	N/A

It appears that the substrates that are amenable to this transformation give nearly identical yields. There are no clear trends in the hydrolysis results, but it is indicative that the identity of the halogen species is not essential for this reaction. One additional result that was interesting was that a small amount of by-product was isolated from the hydrolysis of the cyclohexylmethyl and phenyl analogues (4% for both compounds).

These by-products appeared to have three protons attached to heteroatoms (with some kind of tautomerism occurring in solution) and a molecular mass that was in agreement with the

amino group of the product reacting with one molecule of formic acid to give the proposed compound below (Scheme 52). Unfortunately, despite a great many attempts to characterise these compounds *via* different NMR techniques and at higher fields, the structures of these compounds were not able to be fully elucidated. However, MS does support these conclusions. The incomplete data is included in the experimental section.

If we consider that we have already commented that diaminated compounds can be ring-closed with carboxylic acid derivatives (see Section 2.5), including formic acid,[28] the suggestion that formic acid reacts with the free amino group in this reaction is unsurprising. If one desired to remove this possibility, another type of acid could be utilised.



8 **3** **47** **48**

c: R = cyclohexylmethyl
d: R = phenyl

Scheme 52. Mechanism of creation of a possible by-product of the hydrolysis of compounds **8c** and **8d**.

4.6. Conclusions

Treatment of these four 9-substituted adenines and one 9-substituted 6-chloropurine shows that this method gives moderate yields for the halogenation reaction (60 – 70%) and good to excellent yields for the hydrolysis step. It also shows that choice of the halogen donor is important and some donor may cause the production of unwanted by-products or more difficult purification of the crude product. Hydrolysis works well for both chlorinated purines and brominated purines. Compared to brominating with liquid bromine and NBS, this method gives comparable yields and has a shorter reaction time. However, the requirements of inert atmosphere, low temperatures and aqueous work-up result in some additional work for halogenation *via* lithiation.

CHAPTER 5

5. BIOLOGICAL TESTING

5.1. General

Testing of the four target compounds has been carried out at were at the Institute for Clinical Biochemistry and Institute for Medical Microbiology at the National Hospital in Oslo. At the time of writing, testing had been partly completed and this involved assays with the enzyme human alkyladenine DNA glycosylase (hAAG). Testing is also planned for assays with the enzyme human 8-hydroxyguanine DNA glycosylase (hOGG1). The compounds and some of the intermediates were also tested for some viral activity at a different institute.

5.2. Testing on Human DNA Glycosylases

As mentioned earlier, DNA glycosylases are enzymes that are responsible for identifying and excising specific lesion basses from the DNA strand. The two glycosylases that are most likely to identify our target molecules as damaged bases and show inhibitory effects are hAAG and hOGG1. Human alkyladenine DNA glycosylase (hAAG) (Figure 22) is a human enzyme which recognises damaged bases in DNA and partakes in base excision repair of a diverse selection of substrate bases including alkylated adenines, hypoxanthine and etheno bases.[7,12,13] It was decided to test for inhibition of this enzyme because the molecules made in this project were alkylated adenine-derivatives.

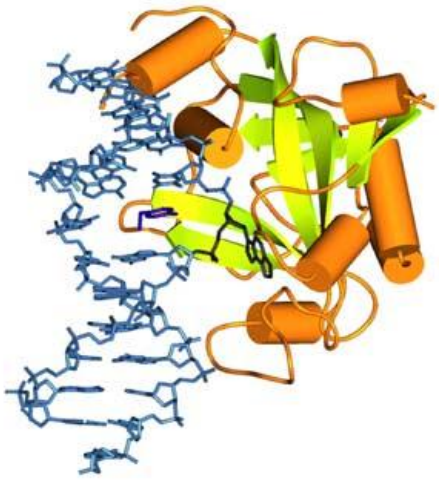


Figure 22. Crystal structure of a hAAG complex with DNA. The base (shown in black) is being flipped into the active site of the glycosylase.[7]

None of the four compounds tested displayed any visible inhibitory effect on enzyme activity of hAAG at 1 mM concentration. DNA with inosine was used as the substrate for the testing and the same amount of cutting of the DNA was observed to be carried out by the enzyme with 1 mM concentration of the compounds as the control test with only the enzyme present.

Recently, the crystal structure of hAAG bound to 3,N^4-ethenocytosine lesions has been elucidated. It has been found that this particular lesion inhibits the activity of the enzyme because hAAG fails to activate the nucleotide base as an efficient leaving group and carry out excision. The structure of the active site of the enzyme from this study could provide interesting information for the design of hAAG inhibitors in the future.[90]

Human 8-hydroxyguanine DNA glycosylase (hOGG1) the main DNA glycosylase that excises the oxidative lesion, 8-oxoguanine, and initiates base excision repair. Since 8-oxoguanine is a common lesion is known to cause mismatches, for example with adenine, and results in mutations, this repair enzyme is extremely important for cell repair. This also makes it a good target for inhibition of repair in cancer cells.[91,92]

It has also been reported that this enzyme is responsible for the repair of some 8-oxoadenine lesions depending on whether they are pair with a cytosine or guanine base.[8] The identification of 8-oxoadenine and 8-oxoguanines is the reasons that this enzyme has been chosen for biological testing.

5.3. Virus Testing

Some of the intermediates and final compounds were also tested for viral activity against a broad panel of viruses. Unfortunately, there were no hits among the compounds synthesised in this thesis. The compounds tested were the four target molecules, 3- and 9-benzyl-8-bromoadenine and 3-benzyl-8-oxoadenine. The viruses for which assays were carried out are listed in Appendix 2).

CHAPTER 6

6. CONCLUSIONS AND FURTHER WORK

6.1. General

The development of medicinal drugs to promote the selective effectiveness of cancer treatment through exploitation of features specific to cancer cells can be a key to the success of the overall cancer therapy. By targeting the actions of specific enzymes that carry out repair on lesions created on DNA in cancer cells, the ability of these cells ability to survive radiation therapy or chemotherapy can be reduced or removed completely. The focus of this project was to synthesise the target molecules for testing, employing various synthetic strategies, which has been accomplished. A summary of the overall yields for all routes discussed in the current project is shown in Table 15.

Table 15. Overview of the best overall results from the syntheses carried out.

Entry	R	Strategy 1 (Br_2, RX, O)	Strategy 2 (Br_2, O, RX)	Strategy 3 (RX, Br_2, O)	Strategy 4 (from pyrimidine)	Strategy 5 (from 6-chloropurine)	Strategy 6 (RX, LDA, O)	**Best overall yield (Strategy)**
1	Benzyl (**3a**)	9	6	**19**	N/A	N/A	-[a)]	**19 (3)**
2	Cyclopentyl (**3b**)	N/A	12	37	N/A	N/A	**46** ($C_2Br_2Cl_4$)	**46 (6)**
3	Cyclohexyl-methyl (**3c**)	-[b)]	12	42	N/A	N/A	**50** ($C_2Br_2Cl_4$)	**50 (6)**
4	Phenyl **(3d)**	N/A	N/A	N/A	**50**	33 (C_2Cl_6)		**50 (4)**

Br_2: bromination with liquid bromine, RX: alkylation with alkyl halide and base, O: acid-catalysed hydrolysis, LDA: lithiation and capture with C2X2Cl4. a) debenzylation observed in halogentaion step; b) insufficient material obtained after second (alkylation) step to complete the scheme.

The synthetic routes used have involved an investigation of the possibilities of functionalising commercially-available purines, adenine and 6-chloropurine, and formation of the purine ring system from commercially available 4,6-dichloro-5-aminopyrimidine. The main challenges met during the project were encountered in the alkylation and arylation steps and factors that contributed to this include the polarity of the adenines increasing the difficulty of separation in purification and the regioselectivity of the alkylation steps.

Good overall yields were obtained for 3b and 3c through Strategy 6 (alkylation, treatment with LDA and dibromotetrachloroethane and hydrolysis). Strategy 3 also gave yields which were not much worse, which indicates that the difference in the bromination step is small since the first and third steps are the same in these two strategies. For compound 3d, the best strategy was that starting from a pyrimidine derivative (Strategy 4). Despite the route having 4 steps, the overall yield was still reasonably good. Compound 3a fared the worst of the analogues with an overall yield of 19%, also from Strategy 3.

Strategy 4 is perhaps the synthesis route which could be interesting to pursue – for example, if we desired higher overall yields for compounds **3**. Following the pathway of aminating 4,6-dichloro-5-aminopyrimidine, treatment with CDI and amination, seems to be an elegant method to regioselectively obtain the target molecules by taking advantage of symmetry in the amination step.

If further analogues were to be synthesised for biological testing, it could be interesting to synthesize analogues with substituents on the aromatic rings or perhaps non-ring substituents. The current thesis has provided some exploration of the possibilities of obtaining 9-substituted-8-oxoadenines while obtaining the target molecules for biological testing.

Another future prospect for this project is the syntheses of 9-substituted-8-oxoguanines for biological testing and it is hoped that some of the results and methods obtained in the course of this project can be carried on to facilitate smoother synthesis of these compounds. In addition, as mentioned in Section 3.1, it could be an interesting, separate project to carry out a thorough survey of the alkylating patterns of different 8-substituted adenines or purines, with a variety of alkylating agents, to gain an overview of substitution patterns and give further insight into the possibilities of functionalising the purine ring.

CHAPTER 7

7. EXPERIMENTAL

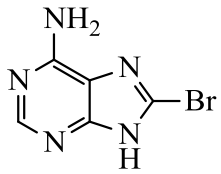
The ^{1}H NMR spectra were recorded at 600 MHz with a Bruker AV 600, at 500 MHz with a Bruker Advance DRX 500 instrument, at 400 MHz with a Bruker DPX 400, 300 MHz with a Bruker DPX 300 or at 200 MHz with a Bruker DPX 200 instrument. The ^{13}C NMR spectra were recorded at 150, 125, 100 or 75* MHz using the above-mentioned instruments. Mass spectra were recorded on a VG Prospec sector instrument from Fissions Instrument at 70 eV ionizing voltage and are presented as *m*/*z* (% rel. int.).

DMF and THF were obtained from a solvent purification system, MB SPS-800 from Mbraun, before each reaction and TMEDA was distilled over KOH. Ethyl acetate, hexanes and dichloromethane were distilled before use. All other reagents were commercially available and used as received. *n*-BuLi was titrated with menthol using 1,10-phenanthroline as an indicator. Diisopropylamine was stirred over sodium hydroxide for at least 24 h, vacuum-distilled over calcium hydride and stored over molecular sieves. Lianne Hill, School of Chemistry University of Birmingham, England, carried out the elemental analyses.

Melting points were determined with a Büchi melting point B-545 apparatus and are uncorrected. Some of the by-products were obtained only impure or in an insufficient amount to carry out melting point measurements. These are labelled “not obtained”.

* In all ^{13}C spectra taken at 75 MHz, there is a peak that is positive and negative, present at approximately 79-80 ppm. This is due to noise from the NMR instrument.

8-Bromo-*9H*-purin-6-amine (5)



5

Adenine (**4**) (5.265 g, 38.96 mmol) and liquid bromine (15 mL) was kept in a closed flask for 4 h, before the stopper was removed. The mixture was allowed to stand overnight, before the resulting solid was stirred in water (50 mL). Concentrated aqueous ammonia was added until dissolution and the solution neutralised with acetic acid. The precipitate was filtered, washed with water, then boiled in water and filtered while hot (washed with water, acetone and ether), then dried *in vacuo*. Yield 6.201 g (74%) beige solid.

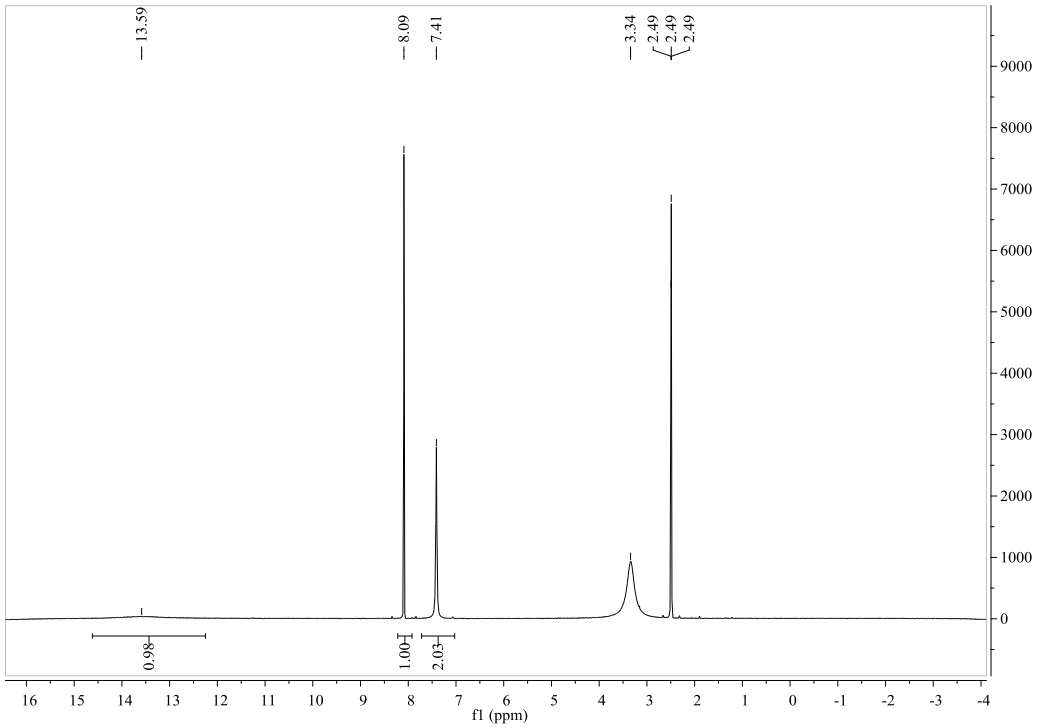
^{1}H NMR (DMSO-d_6, 300 MHz): δ 13.59 (br s, 1H, NH), 8.09 (s, 1H, H-2), 7.41 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 154.0 (C-4 or C-6), 152.1 (C-4 or C-6), 150.0 (C-2), 127.6 (C-8), 119.9 (C-5).

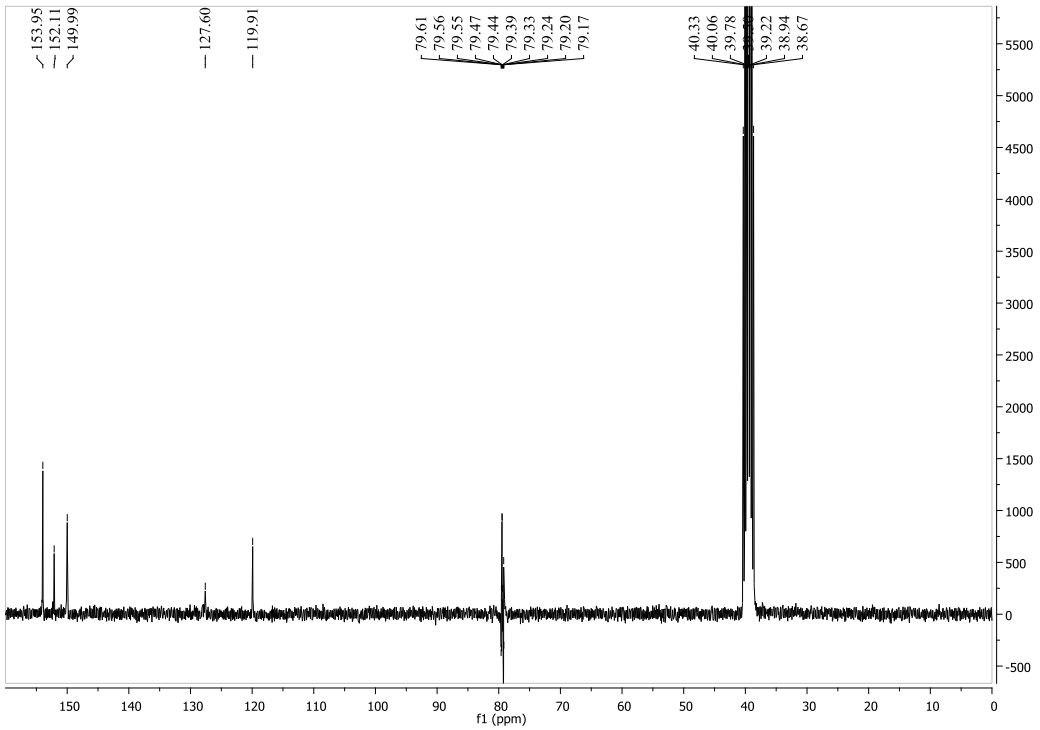
MS EI *m/z* (rel. %): 215/213 (100/97, M^+), 186/188 (36/35), 107 (27).

HR-MS Found 212.9646, calculated for $C_5H_4N_5Br$ 212.9650.

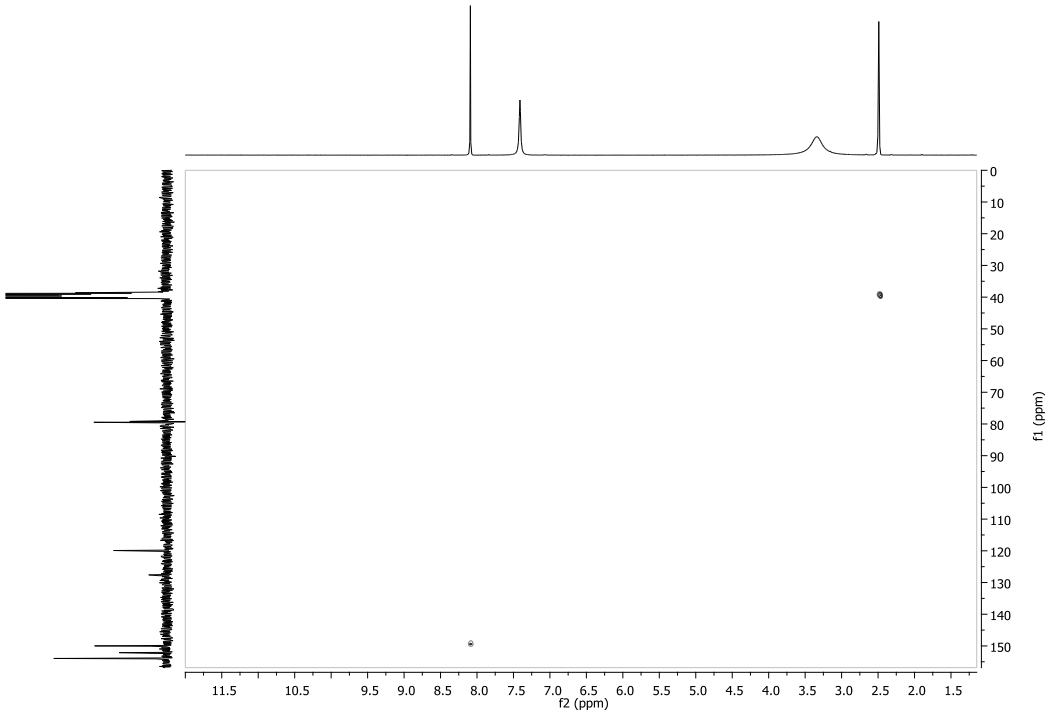
M.p. > 260 ºC (decomposed) (lit.[58] > 250 ºC).



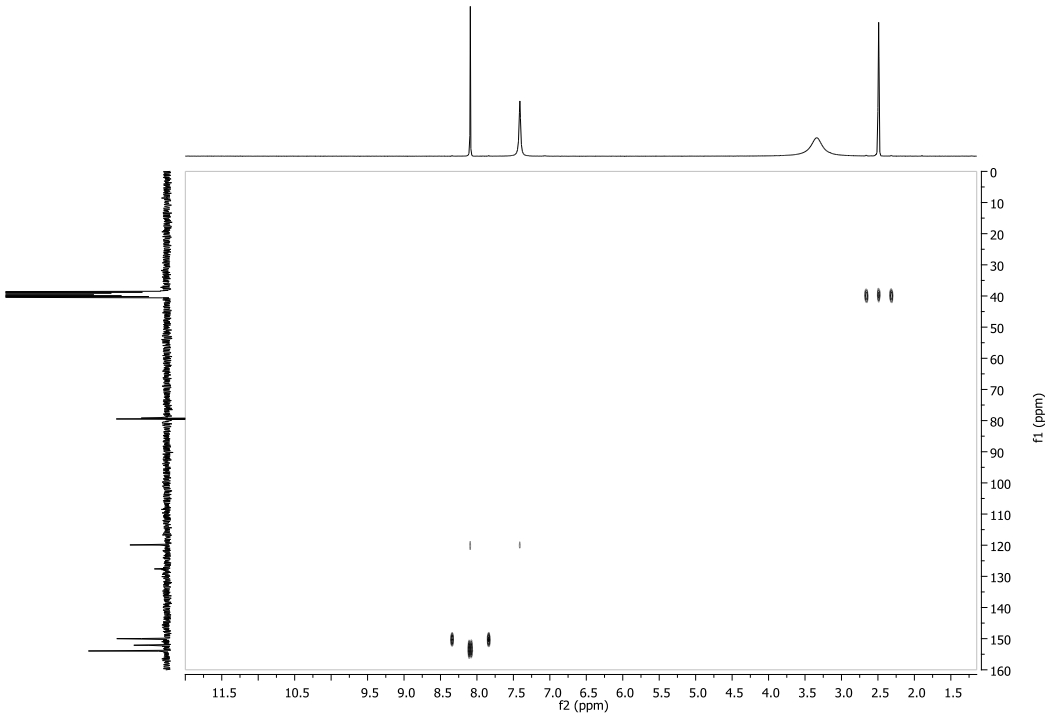
Spectrum 6. ^{1}H NMR of 8-Bromo-*9H*-purin-6-amine (**5**).



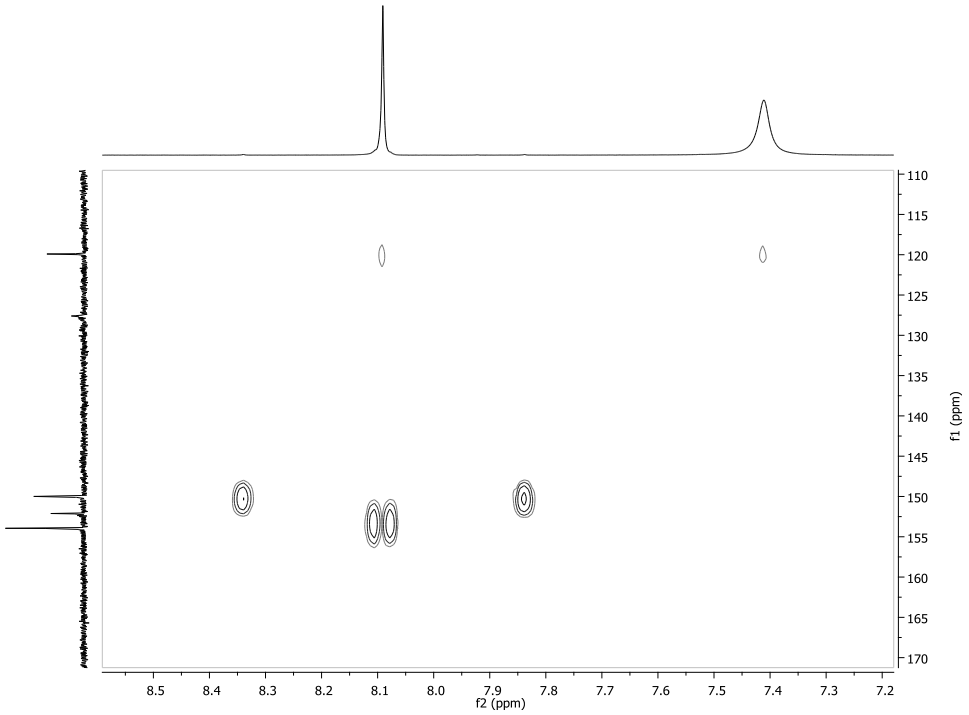
Spectrum 7. ^{13}C NMR of 8-Bromo-*9H*-purin-6-amine (**5**).



Spectrum 8. HSQC of 8-Bromo-*9H*-purin-6-amine (**5**).

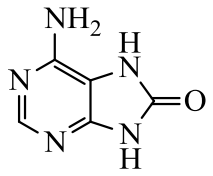


Spectrum 9. HMBC of 8-Bromo-*9H*-purin-6-amine (**5**).



Spectrum 10. HMBC of 8-Bromo-*9H*-purin-6-amine (**5**), expansion of the aromatic region.

6-Amino-*7H*-purin-8(*9H*)-one (2a)



2a

8-Bromoadenine (**5**) (439 mg, 2.05 mmol) was refluxed in formic acid (50 mL) for 24 h and compound **2a** recrystallized from water as a beige powder (190 mg, 58%).

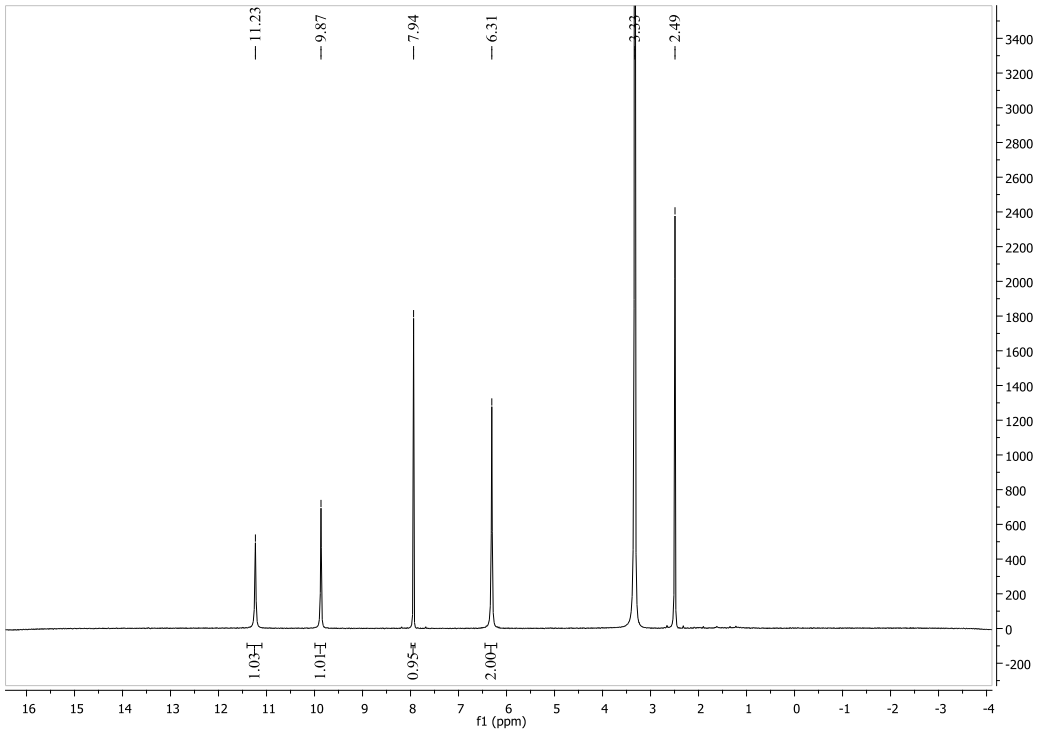
^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.23 (s, 1H, NH), 9.87 (s, 1H, NH), 7.94 (s, 1H, H-2), 6.31 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 100 MHz): δ 152.7 (C-8), 150.8 (C-2), 148.0 (C-4 or C-6), 146.3 (C-4 or C-6), 104.3 (C-5).

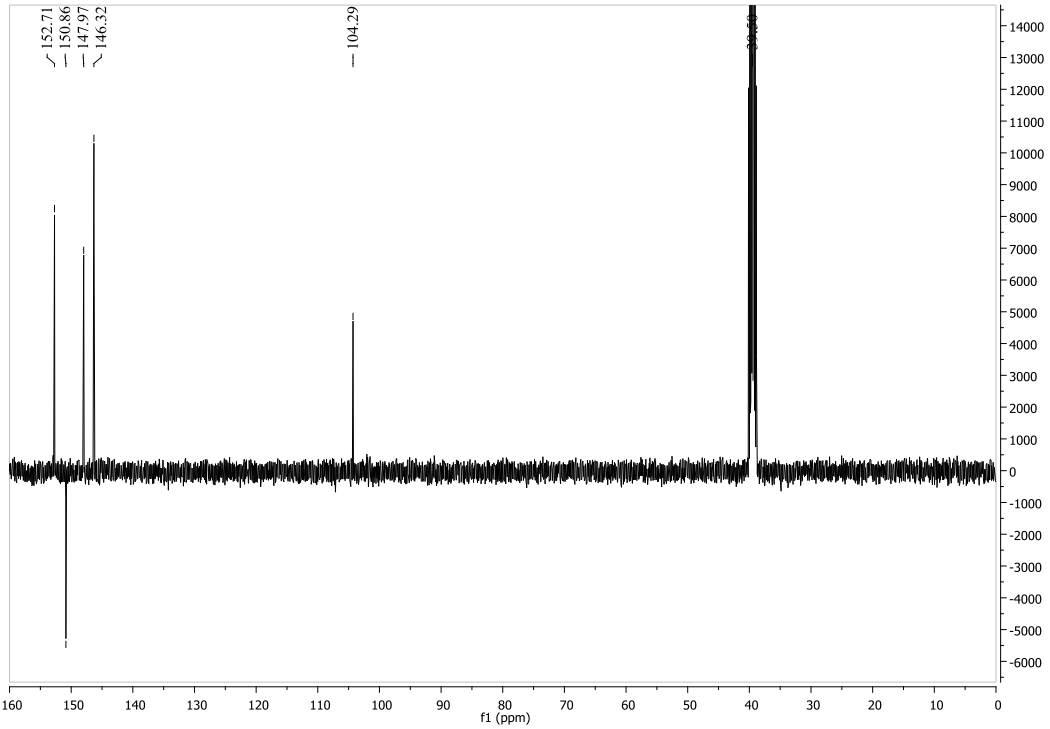
MS EI *m/z* (rel. %): 151 (100, M^+), 124 (7), 97 (6).

HR-MS Found 151.0490, calculated for $C_5H_5N_5O$ 151.0494.

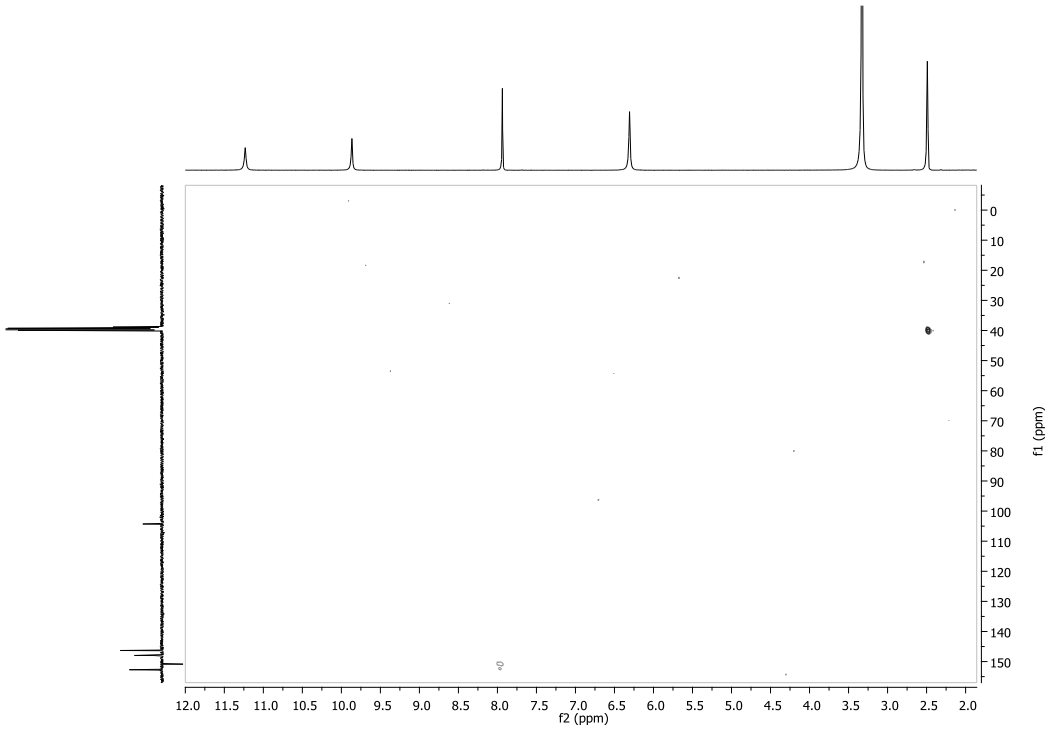
M.p. > 250 °C (decomposed).



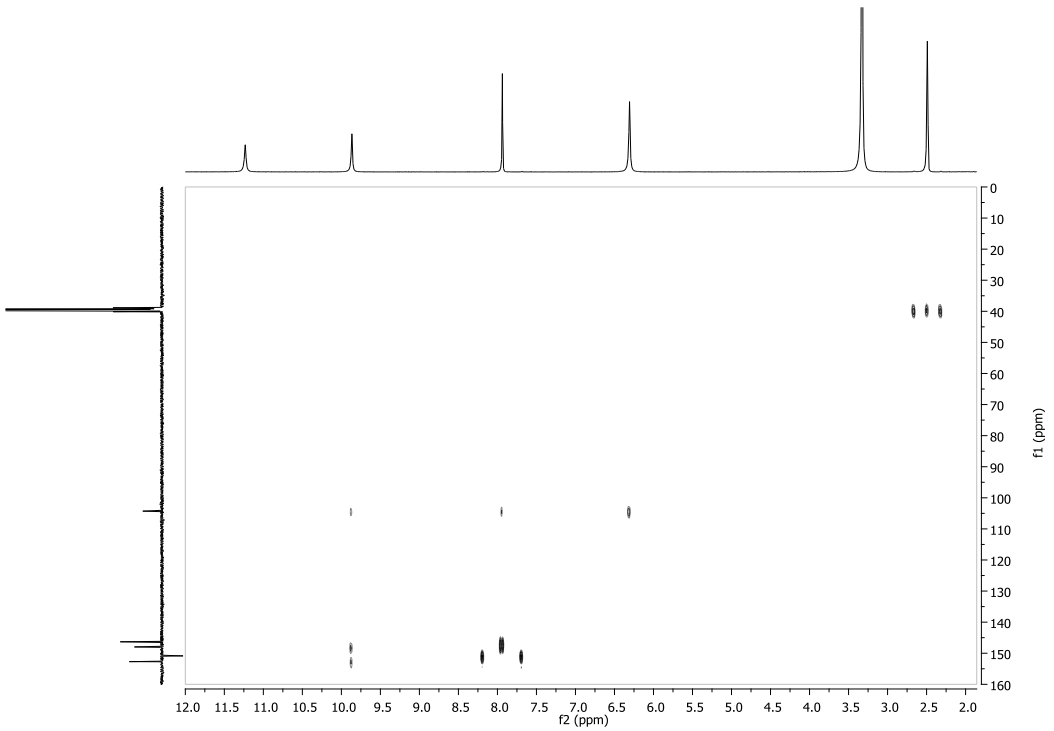
Spectrum 11. ^{1}H NMR of 6-Amino-*7H*-purin-8(*9H*)-one (**2a**).



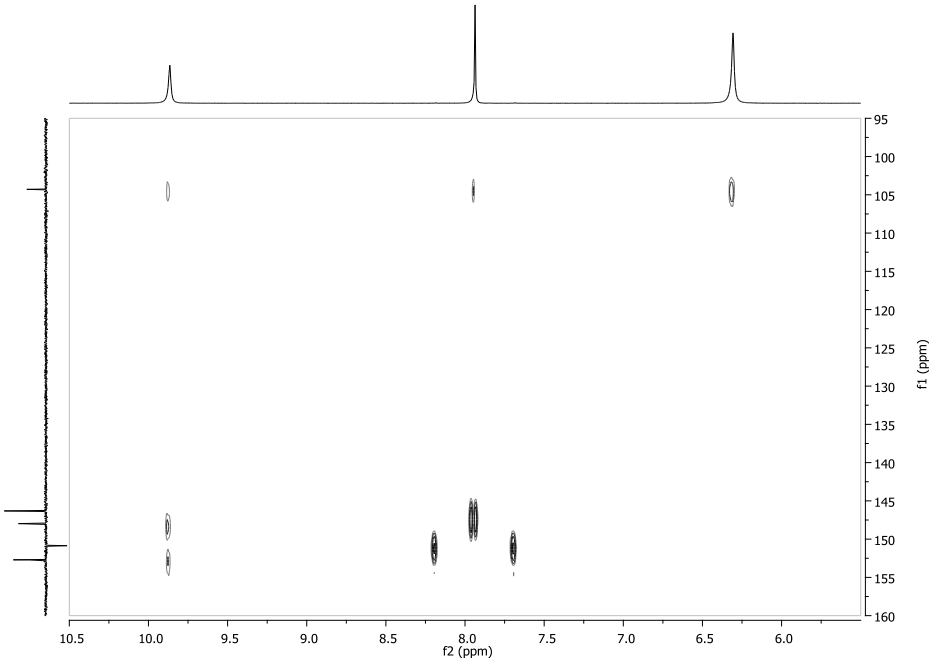
Spectrum 12. ^{13}C APT NMR of 6-Amino-*7H*-purin-8(*9H*)-one (**2a**).



Spectrum 13. HSQC of 6-Amino-*7H*-purin-8(*9H*)-one (**2a**).

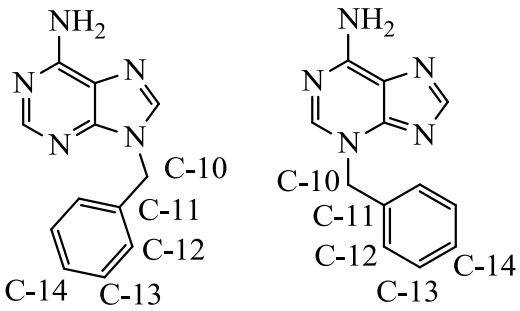


Spectrum 14. HMBC of 6-Amino-*7H*-purin-8(*9H*)-one (**2a**).



Spectrum 15. HMBC of 6-Amino-*7H*-purin-8(*9H*)-one (**2a**), expansion of the aromatic region.

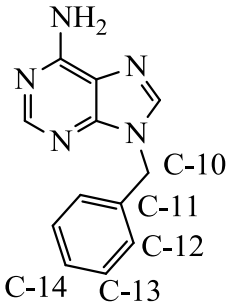
9-Benzyl-*9H*-purin-6-amine (7a) and 3-Benzyl-*3H*-purin-6-amine (28a)



7a **28a**

A mixture of adenine (**4**) (271 mg, 2.01 mmol), DMF (10 mL) and potassium carbonate (552 mg, 3.99 mmol) was stirred under an argon atmosphere. Benzyl bromide (0.30 mL, 2.5 mmol) was added and the mixture stirred at the same temperature for 4 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (0-10% methanol in dichloromethane) to give compounds **7a** (241 mg, 53%) and **28a** (102 mg, 23%) as colourless powders.

9-Benzyl-*9H*-purin-6-amine (7a)



7a

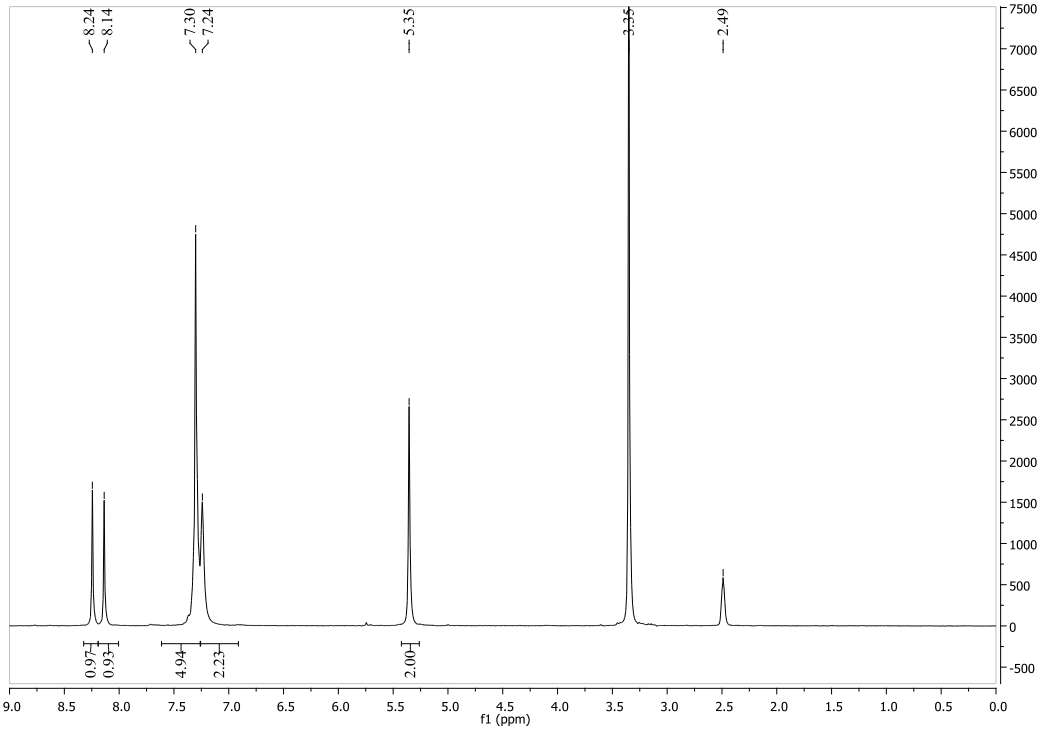
^{1}H NMR (DMSO-d_6, 200 MHz): δ 8.24 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.30 – 7.24 (m, 5H, H-12, H-13 and H-14), 7.24 (br s, 2H, NH_2), 5.35 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 156.0 (C-6), 152.6 (C-2), 149.5 (C-4), 140.8 (C-8), 137.1 (C-11), 128.6 (C-13), 127.7 (C-14), 127.5 (C-12), 118.7 (C-5), 46.1 (C-10).

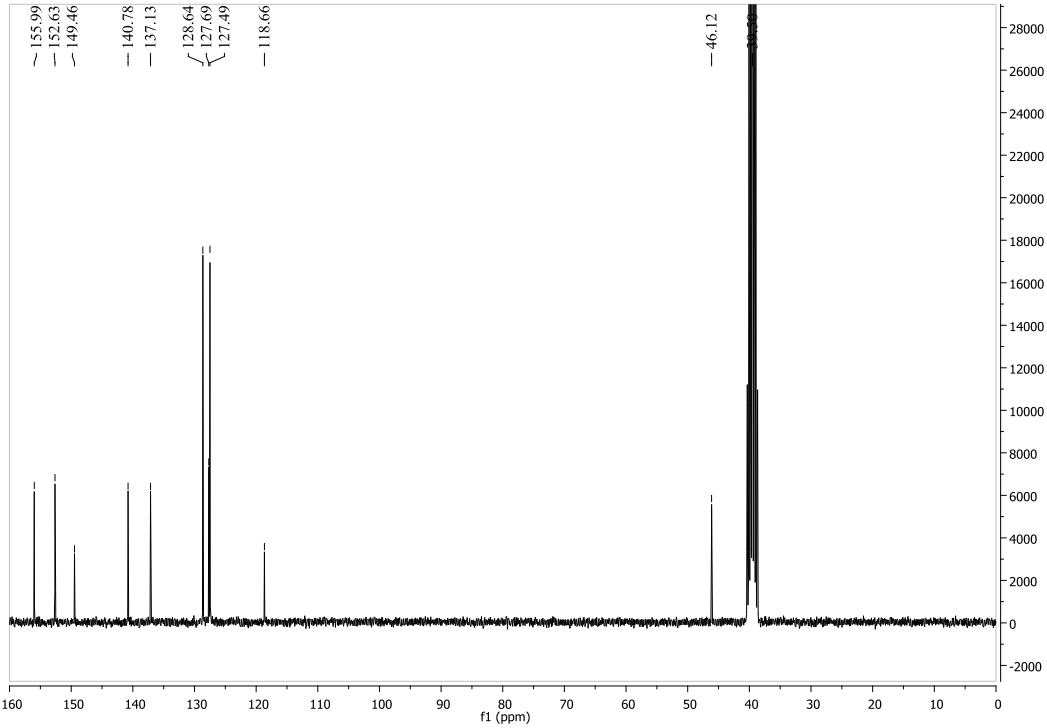
MS EI *m/z* (rel. %): 225 (79, M^+), 224 (100), 182 (15), 148 (8), 91(82), 65 (17).

HR-MS Found 225.1010, calculated for $C_{12}H_{11}N_5$ 225.1014.

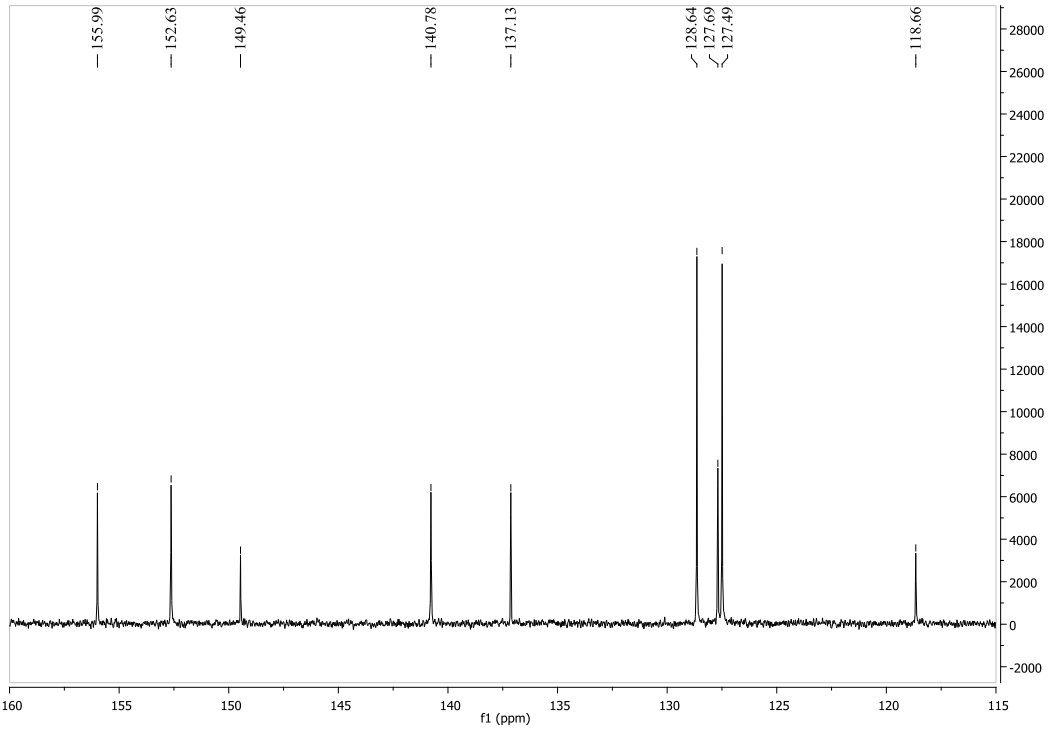
M.p. 233-235 °C (lit.[93] = 231-234 °C).



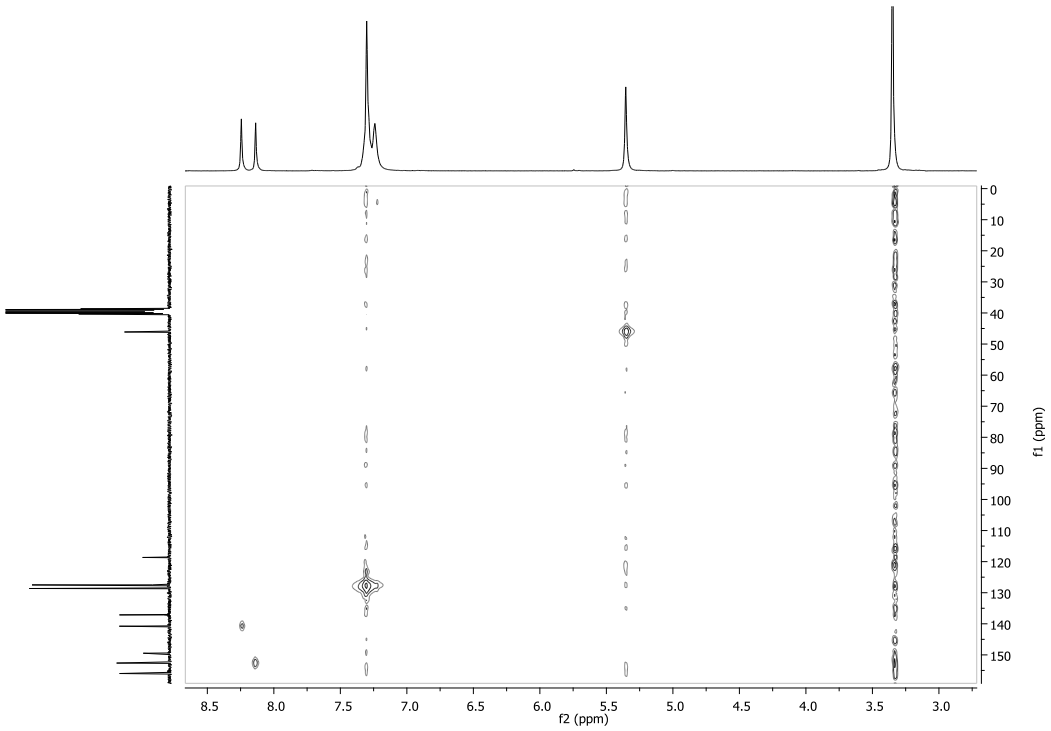
Spectrum 16. ^{1}H NMR of 9-Benzyl-*9H*-purin-6-amine (**7a**).



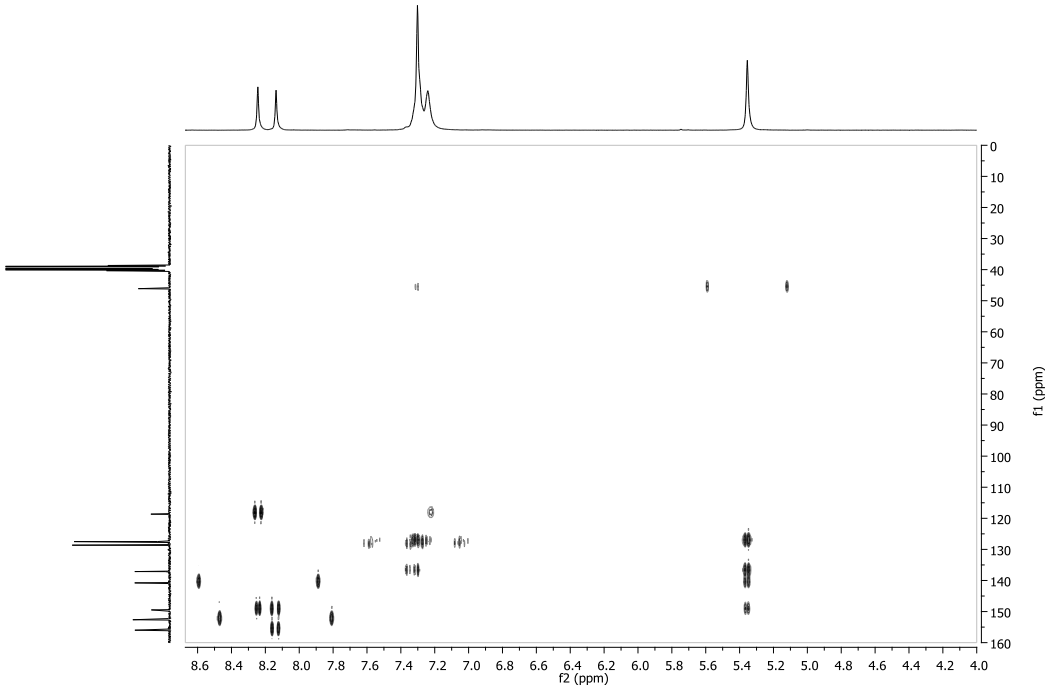
Spectrum 17. ^{13}C NMR of 9-Benzyl-*9H*-purin-6-amine (**7a**).



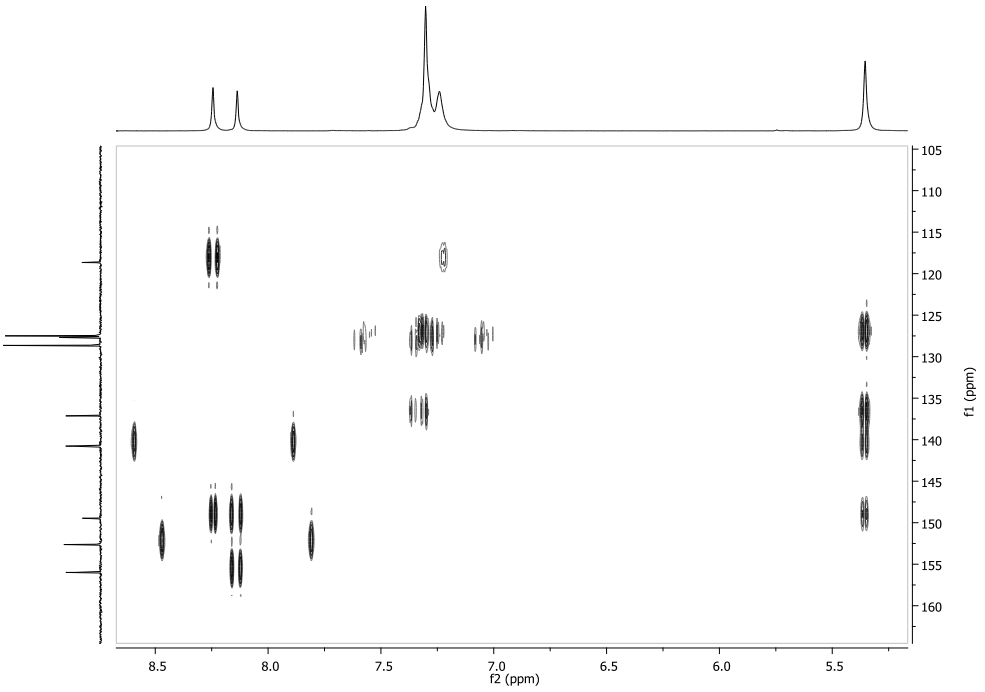
Spectrum 18. ^{13}C NMR of 9-Benzyl-*9H*-purin-6-amine (**7a**), expansion of the aromatic region.



Spectrum 19. HMQC of 9-Benzyl-*9H*-purin-6-amine (**7a**).

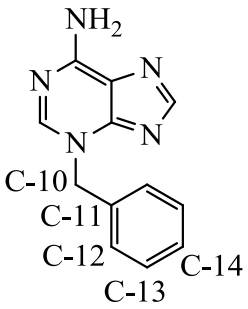


Spectrum 20. HMBC of 9-Benzyl-*9H*-purin-6-amine (**7a**).



Spectrum 21. HMBC of 9-Benzyl-*9H*-purin-6-amine (**7a**), expansion of aromatic and benzylic region.

3-Benzyl-*3H*-purin-6-amine (28a)



28a

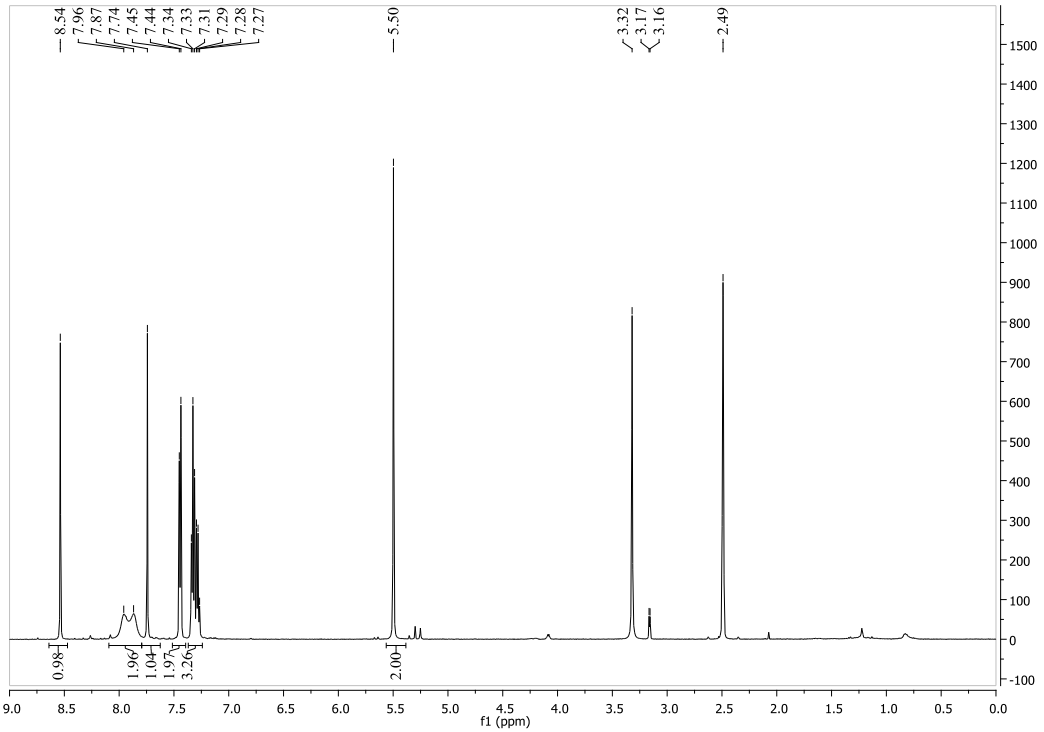
^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.54 (s, 1H, H-2), 7.96 and 7.87 (br d, 2H, NH_2), 7.74 (H-8), 7.45 – 7.44 (d, 2H, H-12), 7.34 – 7.27 (m, 3H, H-13 and H-14), 5.50 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 125 MHz) δ 154.9 (C-6), 152.6 (C-8), 149.7 (C-4), 143.3 (C-2), 136.1 (C-11), 128.6 (C-12 or C-13), 128.04 (C-12 or C-13), 127.99 (C-14), 120.5 (C-5), 52.1 (C-10).

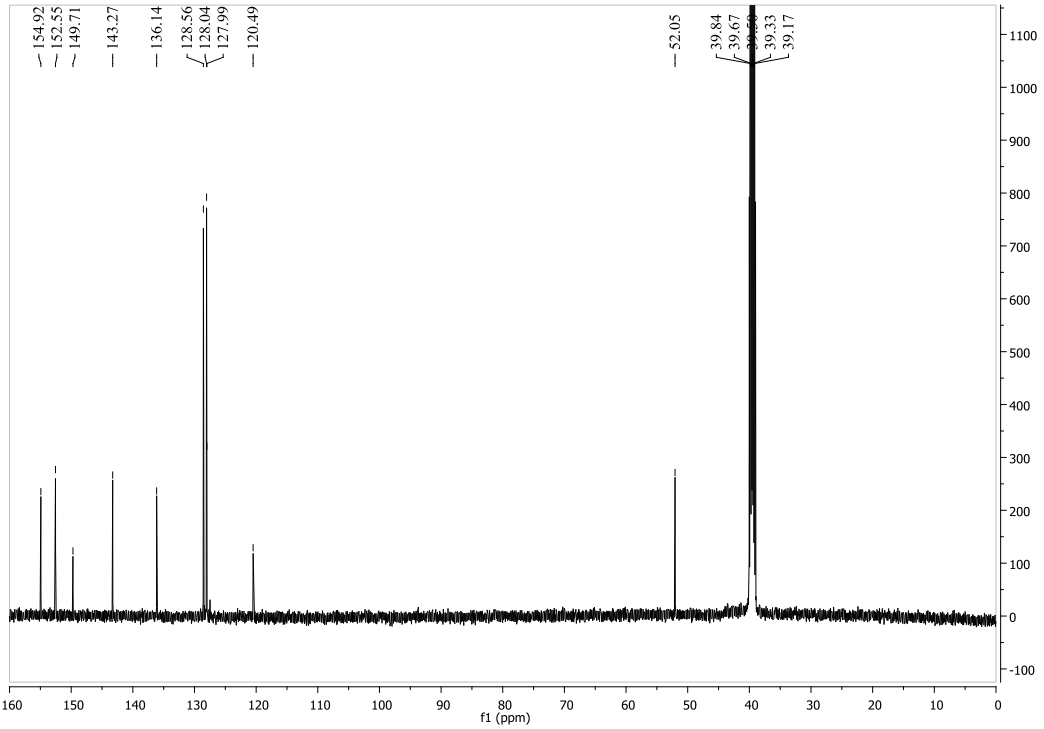
MS EI *m/z* (rel. %): 225 (44, M^+), 224 (100), 91 (72), 65 (12).

HR-MS Found 225.1006, calculated for $C_{12}H_{11}N_5$ 225.1014.

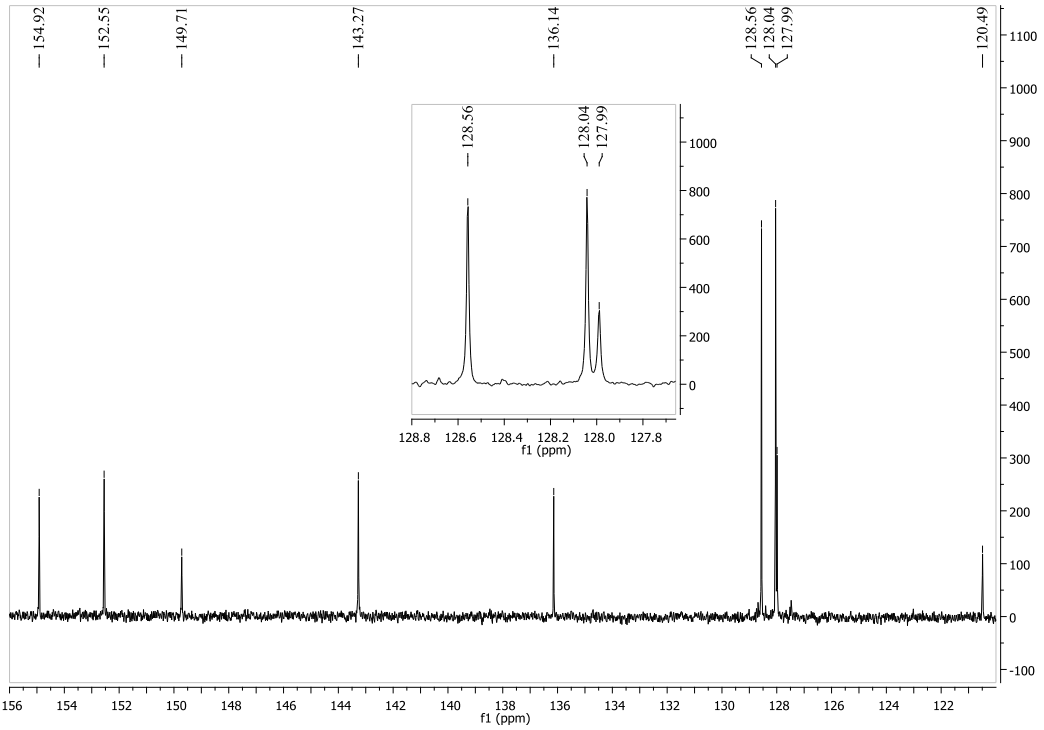
M.p. 265-268 °C



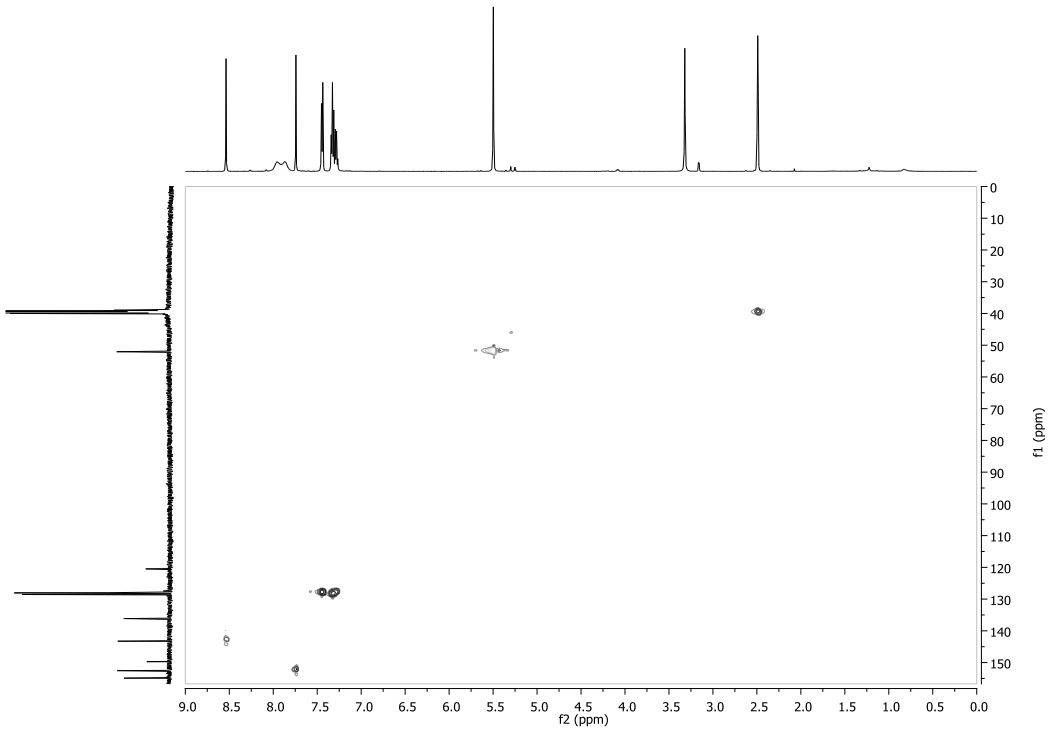
Spectrum 22. ^{1}H NMR of 3-Benzyl-*3H*-purin-6-amine (**28a**).



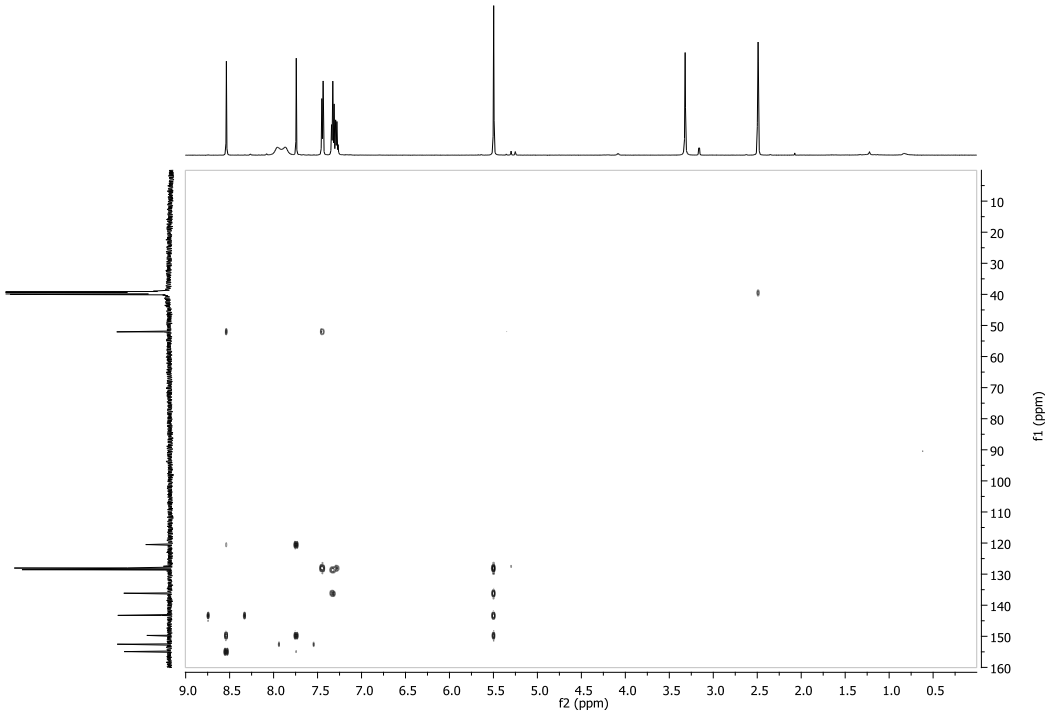
Spectrum 23. ^{13}C NMR of 3-Benzyl-*3H*-purin-6-amine (**28a**).



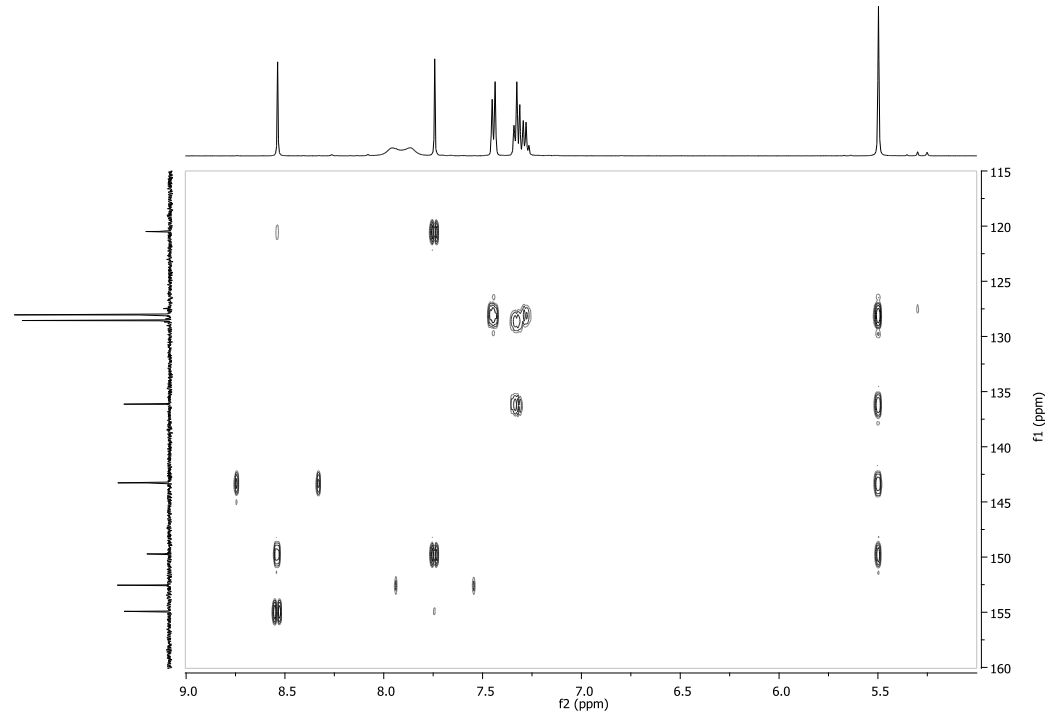
Spectrum 24. ^{13}C NMR of 3-Benzyl-*3H*-purin-6-amine (**28a**), expansion of the aromatic region and expansion of region showing phenyl signals (inset).



Spectrum 25. HSQC of 3-Benzyl-*3H*-purin-6-amine (**28a**).

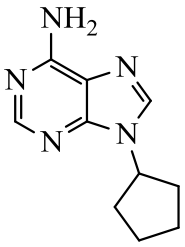


Spectrum 26. HMQC of 3-Benzyl-*3H*-purin-6-amine (**28a**).

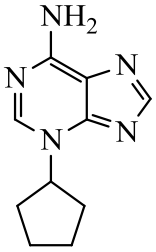


Spectrum 27. HMBC of 3-Benzyl-*3H*-purin-6-amine (**28a**), expansion of the benzylic and aromatic region.

9-Cyclopentyl-*9H*-purin-6-amine (7b) and 3-Cyclopentyl-*3H*-purin-6-amine (28b)



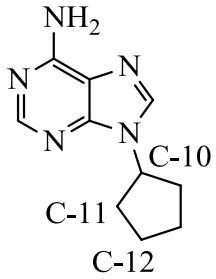
7b



28b

A mixture of adenine (**4**) (548 mg, 4.06 mmol), DMF (20 mL) and potassium carbonate (1117 mg, 8.082 mmol) was stirred at 65 °C under a nitrogen atmosphere for 30 minutes. Bromocyclopentane (0.81 mL, 7.6 mmol) was added and the mixture stirred at the same temperature for another 72 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (0-10% methanol in dichloromethane) to give **7b** as a colourless powder (720 mg, 70%) and **28b** (72.3 mg, 7%, after recrystallization with chloroform/hexanes) as colourless crystals.

9-Cyclopentyl-*9H*-purin-6-amine (7b)



7b

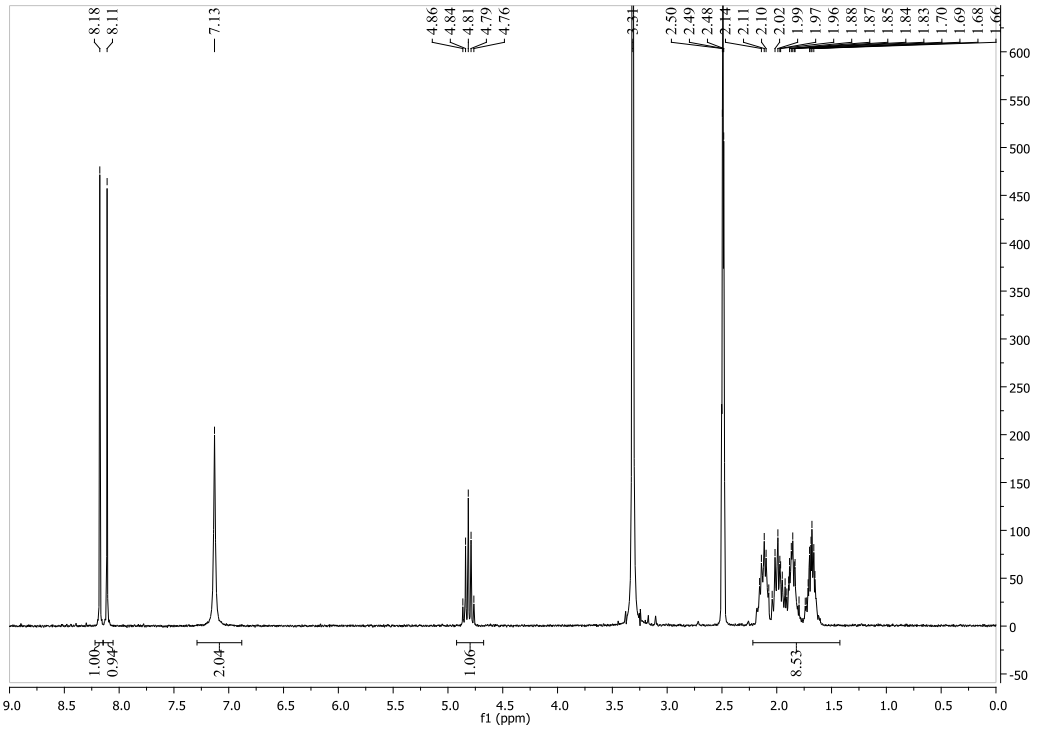
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.18 (s, 1H, H-2), 8.11 (s, 1H, H-8), 7.13 (s, 2H, NH_2), 4.81 (p, *J* = 7.5 Hz, 1H, H-10), 2.22 – 1.42 (m, 8H, H-11 and H-12).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.0 (C-6), 152.2 (C-2), 149.4 (C4), 139.3 (C-8), 119.2 (C-5), 55.3 (C-10), 31.9 (C-11), 23.5 (C-12).

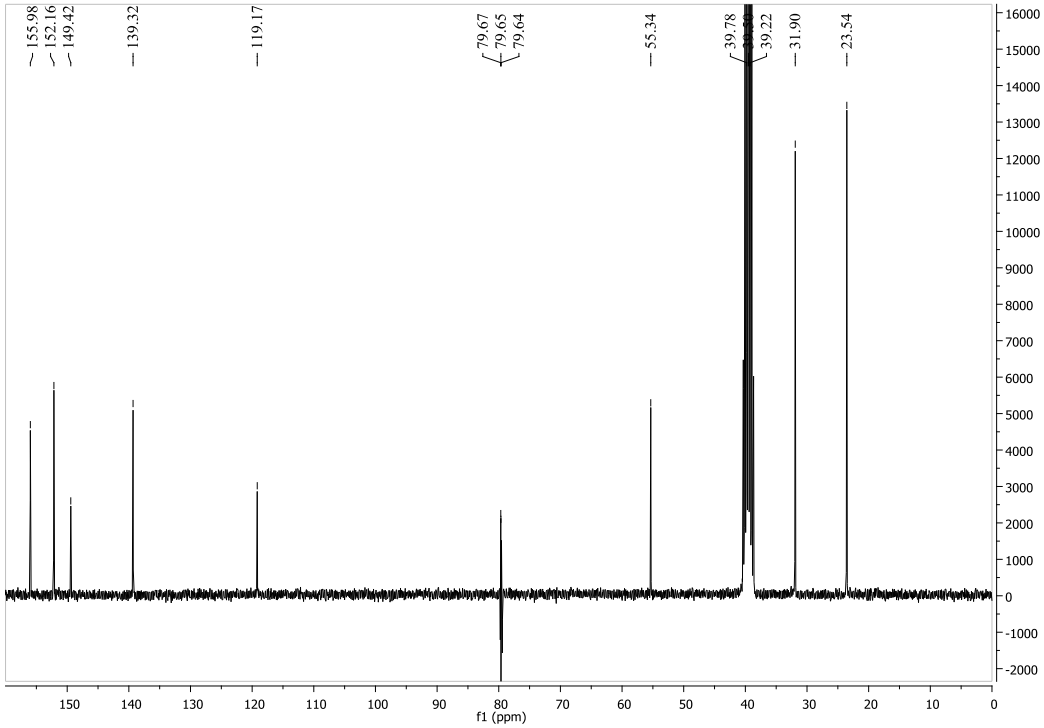
MS EI *m/z* (rel. %) 203 (47, M^+), 162 (48), 135 (100), 108 (36), 67 (7).

HR-MS Found 203.1167, calculated for $C_{10}H_{13}N_5$ 203.1171.

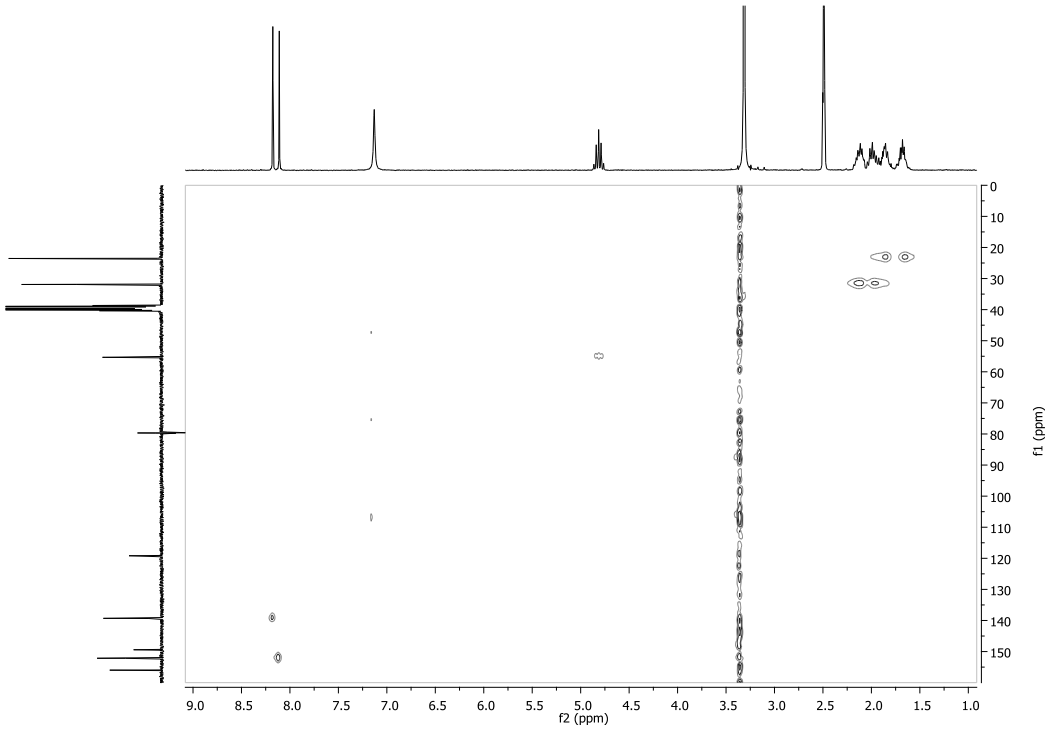
M.p. 155-157 °C (lit.[69] = 154 °C).



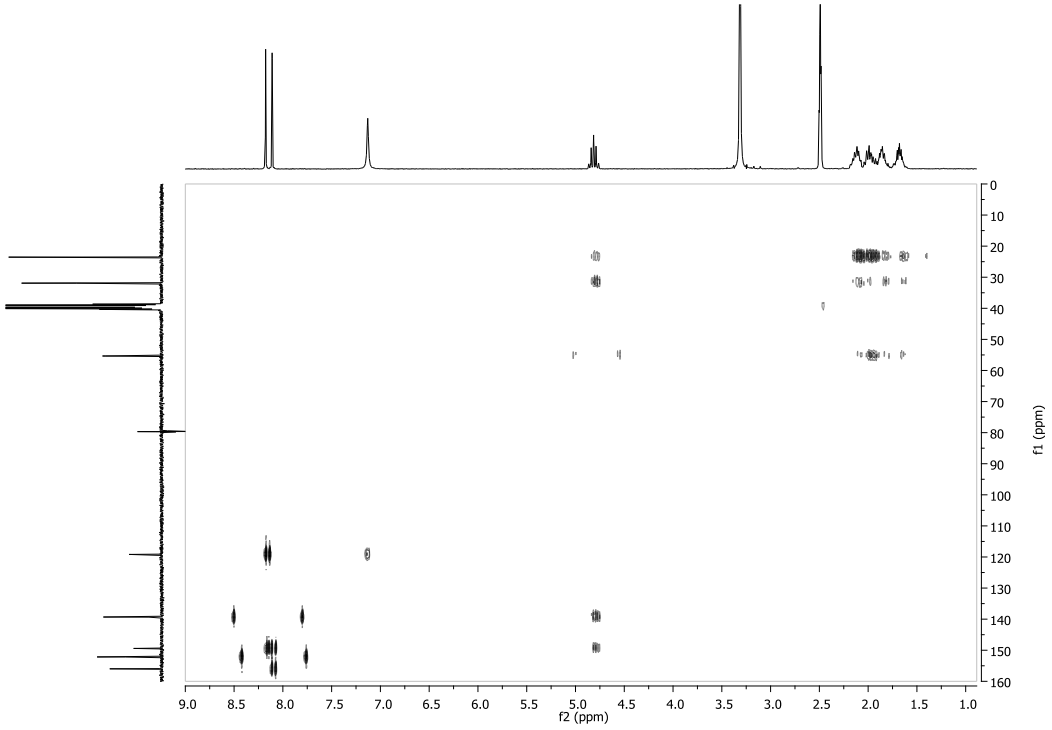
Spectrum 28. ^{1}H NMR of 9-Cyclopentyl-*9H*-purin-6-amine (**7b**).



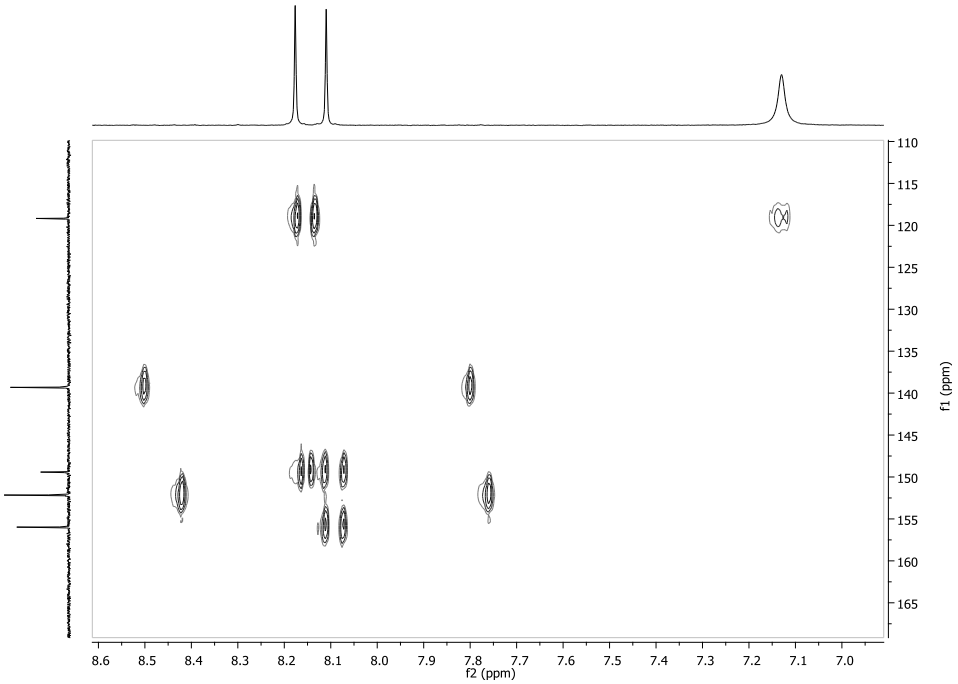
Spectrum 29. ^{13}C NMR of 9-Cyclopentyl-*9H*-purin-6-amine (**7b**).



Spectrum 30. HMQC of 9-Cyclopentyl-*9H*-purin-6-amine (**7b**).

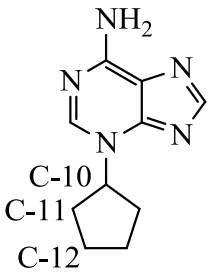


Spectrum 31. HMBC of 9-Cyclopentyl-*9H*-purin-6-amine (**7b**).



Spectrum 32. HMBC of 9-Cyclopentyl-*9H*-purin-6-amine (**7b**), expansion of the aromatic region.

3-Cyclopentyl-*3H*-purin-6-amine (28b)



28b

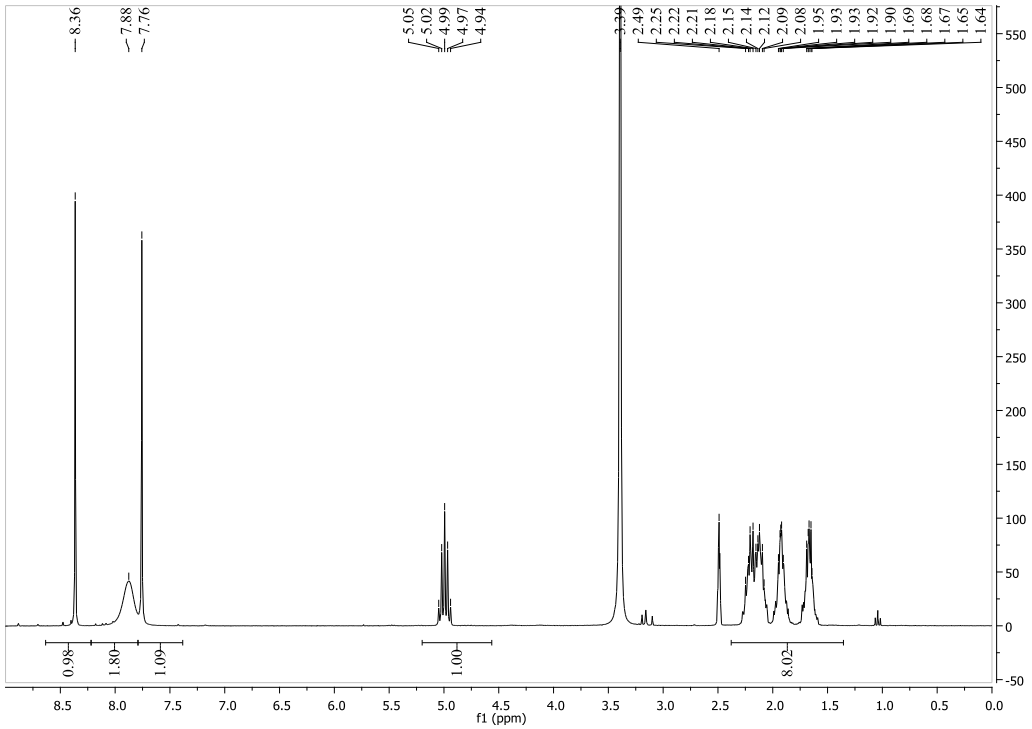
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.36 (s, 1H, H-2), 7.88 (s, 2H, NH_2), 7.76 (s, 1H, H-8), 4.99 (p, J = 8.1 Hz, 1H, H-10), 2.38 – 1.36 (m, 8H, H-11 and H-12).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.7 (C-6), 153.1 (C-8), 150.1 (C-4), 142.9 (C-2), 121.6 (C-5), 62.6 (C-10), 31.5 (C-11), 25.8 (C-12).

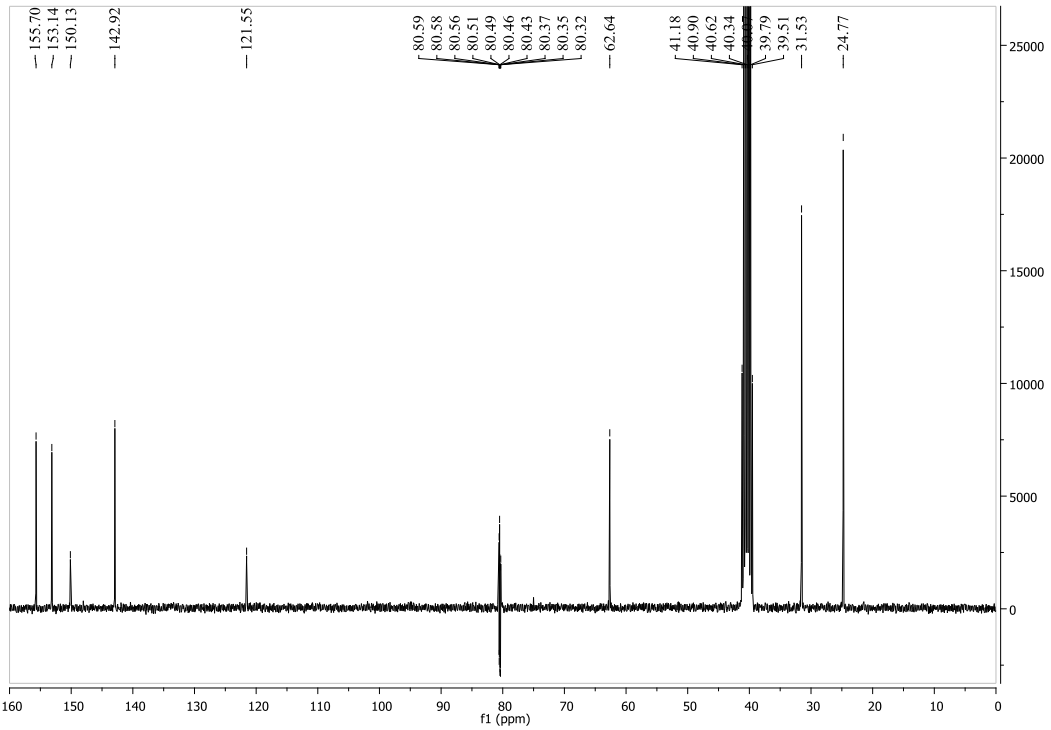
MS EI *m/z* (rel. %) 203 (23, M^+), 162 (30), 135 (100), 108 (28), 67 (7).

HR-MS Found 203.1167, calculated for $C_{10}H_{13}N_5$ 203.1171.

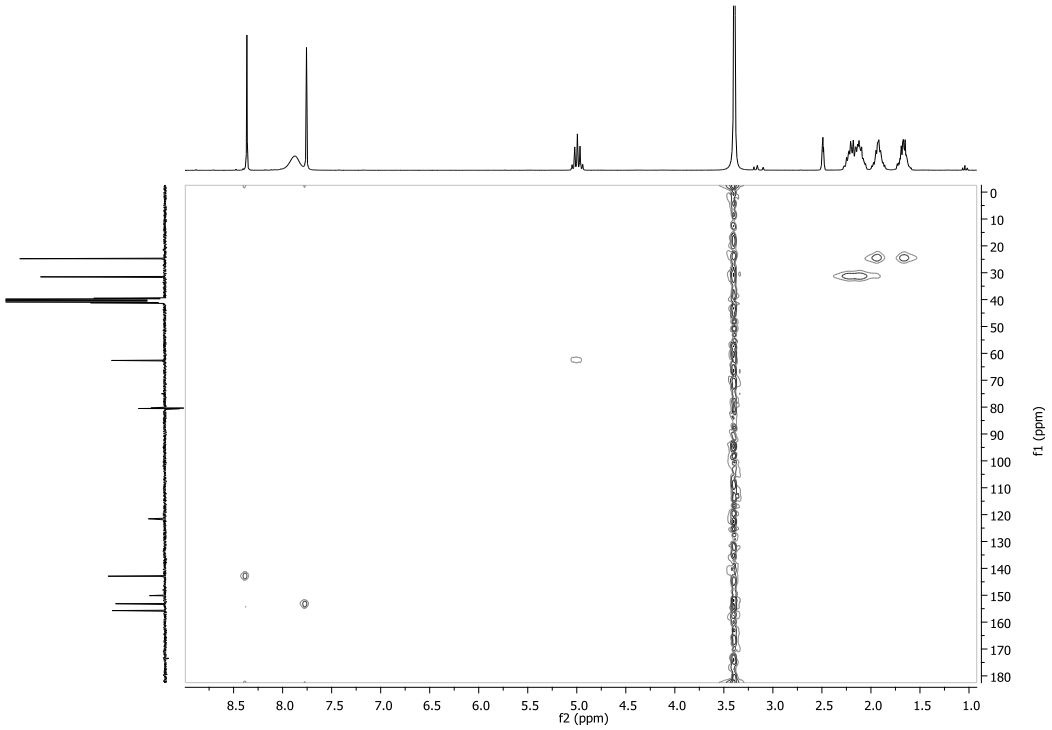
M.p. 209-210 °C.



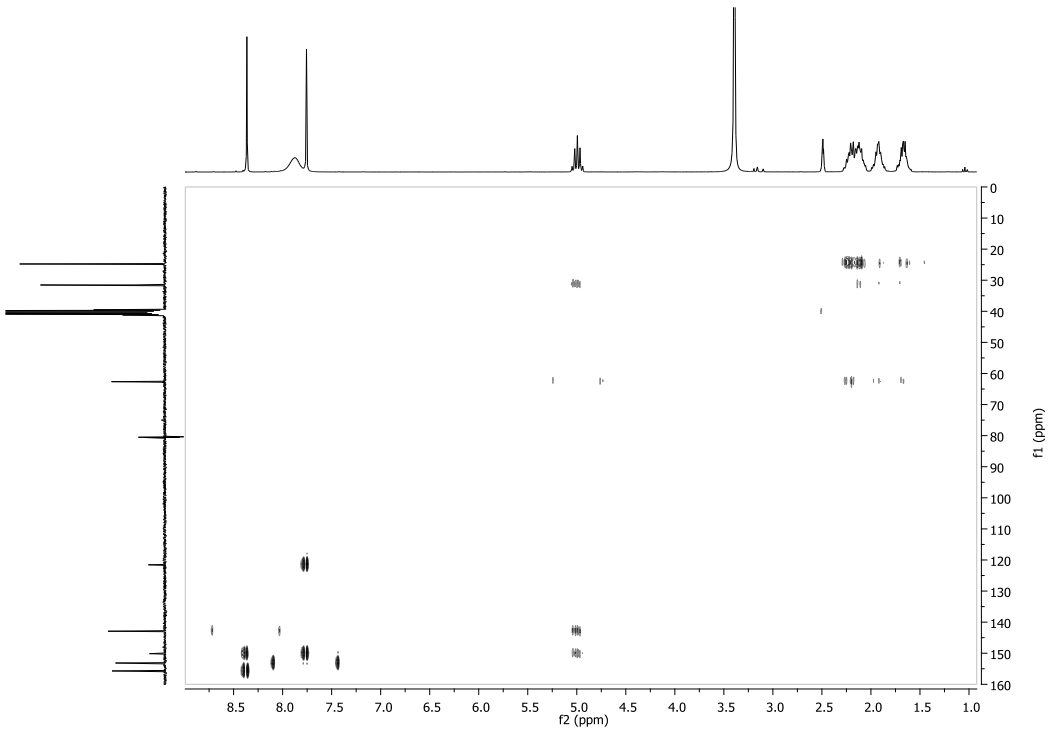
Spectrum 33. ^{1}H NMR of 3-Cyclopentyl-*3H*-purin-6-amine (**28b**).



Spectrum 34. ^{13}C NMR of 3-Cyclopentyl-*3H*-purin-6-amine (**28b**).

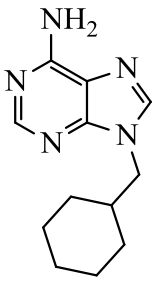


Spectrum 35. HMQC of 3-Cyclopentyl-*3H*-purin-6-amine (**28b**).

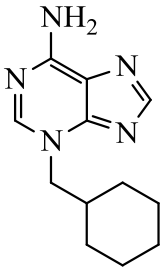


Spectrum 36. HMBC of 3-Cyclopentyl-*3H*-purin-6-amine (**28b**).

9-(Cyclohexylmethyl)-*9H*-purin-6-amine (7c) and 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (28c)



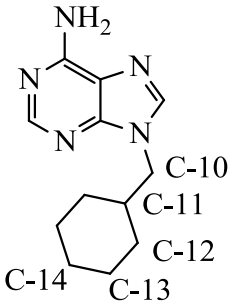
7c



28c

A mixture of adenine (**4**) (135 mg, 1.00 mmol), DMF (5 mL) and potassium carbonate (314 mg, 2.28 mmol) was stirred at 65 °C under a nitrogen atmosphere for 30 minutes. (Bromomethyl)cyclohexane (0.21 mL, 1.5 mmol) was added and the mixture stirred at the same temperature for another 72 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (0-10% methanol in dichloromethane) to give **7c** as a pale yellow powder (165 mg, 71%) and **28c** (30 mg, 13%) as a colourless powder.

9-(Cyclohexylmethyl)-*9H*-purin-6-amine (7c)



7c

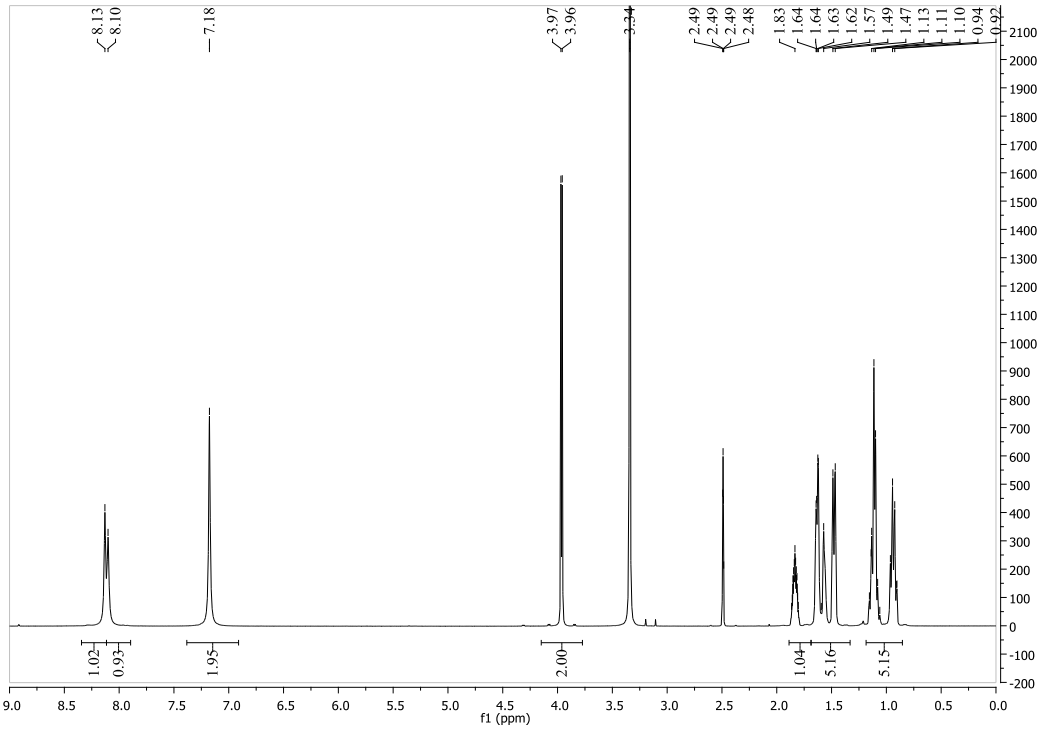
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.13 (s, 1H, H-2), 8.10 (s, 1H, H-8), 7.18 (s, 2H, NH_2), 3.96 (d, J = 7.2 Hz, 2H, H-10), 1.83 (dtt, J = 14.8, 7.4, 3.7 Hz, H-11), 1.64 – 1.47 (m, 5H, H-12, H-13 and H-14,), 1.18 – 0.85 (m, 5H, H-12, H-13 and H-14).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.0 (C-6), 152.3 (C-2), 149.9 (C-4), 141.3 (C-8), 118.9 (C-5), 48.7 (C-10), 37.5 (C-11), 30.0 (C-12), 25.8 (C-14), 25.0 (C-13).

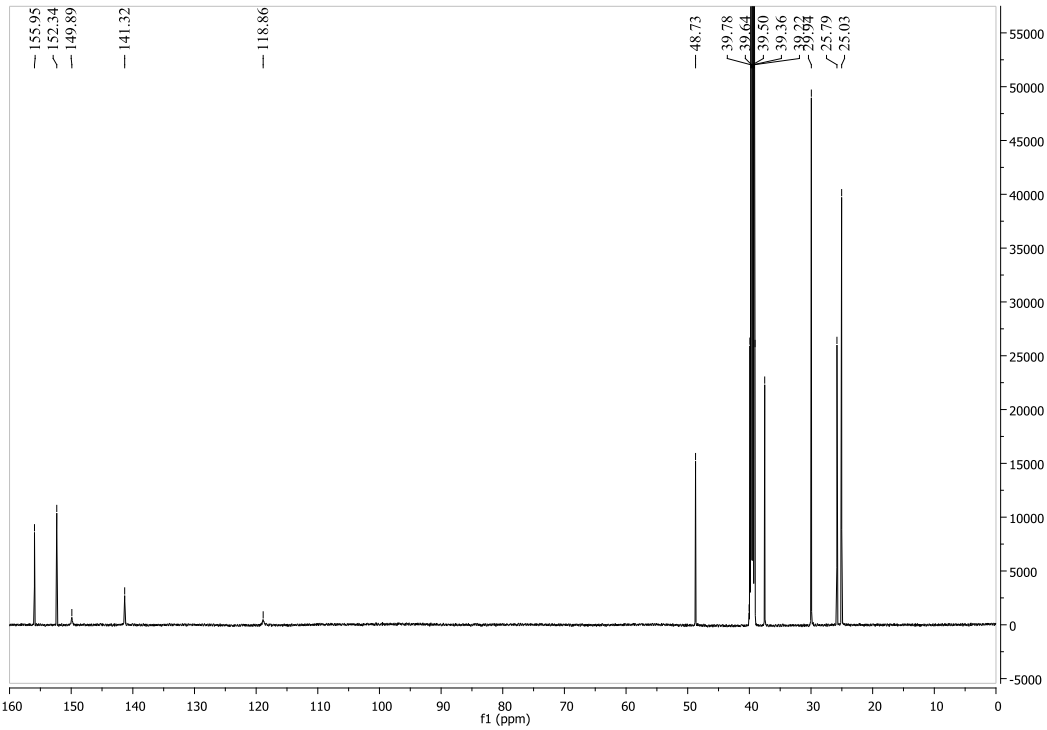
MS EI *m/z* (rel. %) 231 (72, M^+), 188 (12), 149 (100), 148 (76), 136 (22), 135 (80), 108 (15).

HR-MS Found 231.1478, calculated for $C_{12}H_{17}N_5$ 231.1484.

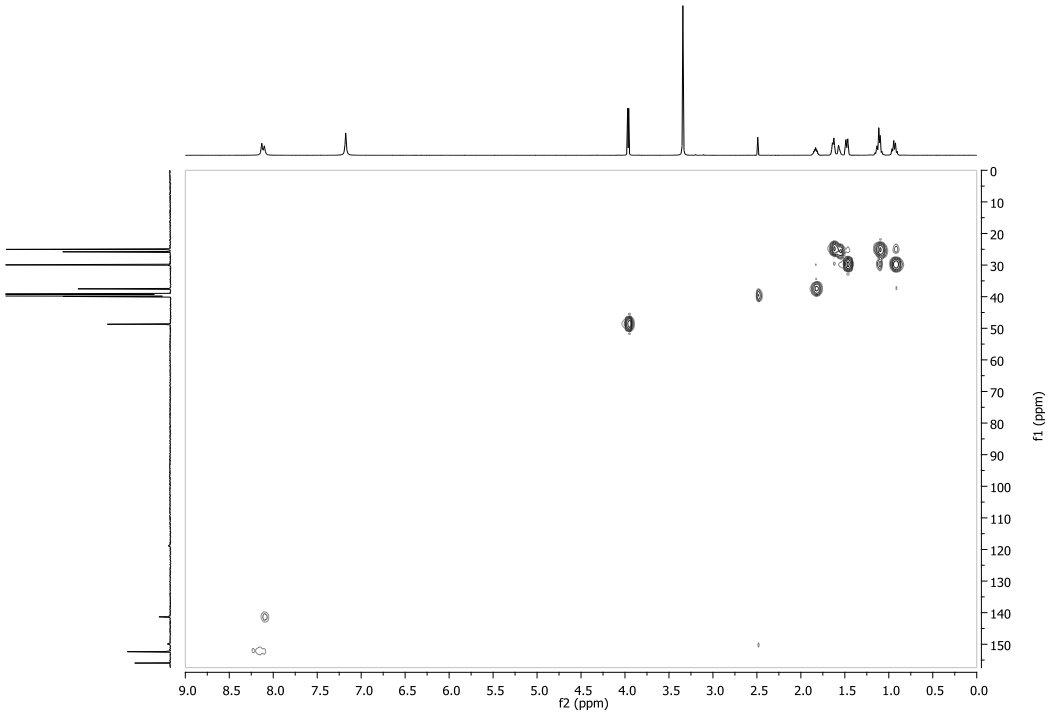
M.p. 224-226 °C.



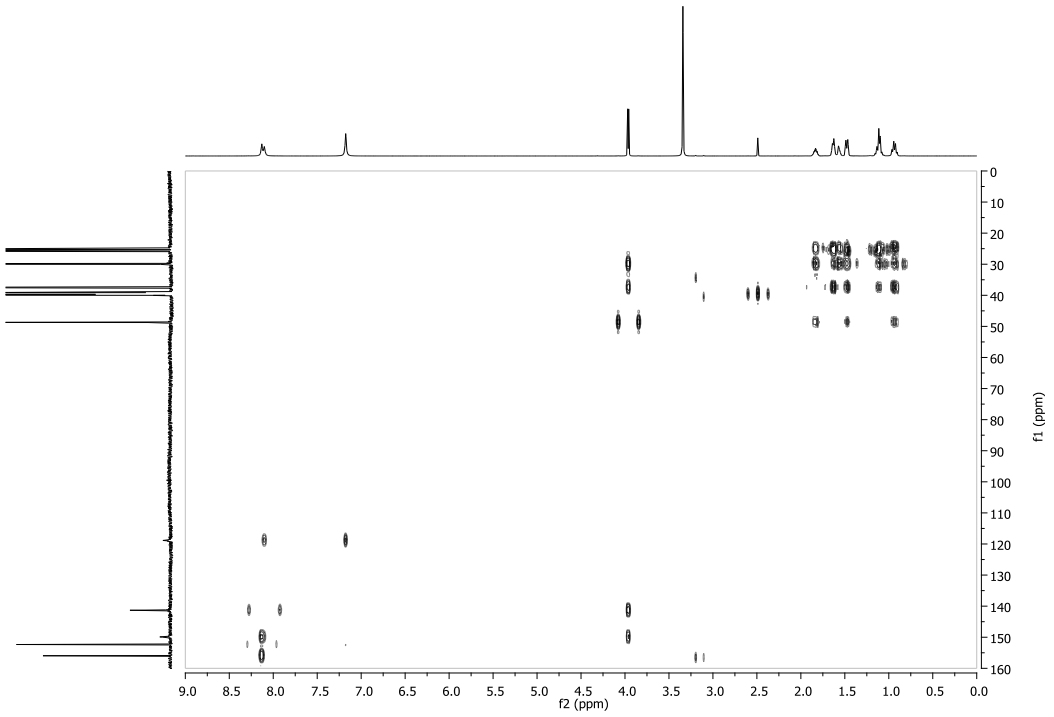
Spectrum 37. ^{1}H NMR of 9-(Cyclohexylmethyl)-*9H*-purin-6-amine (**7c**).



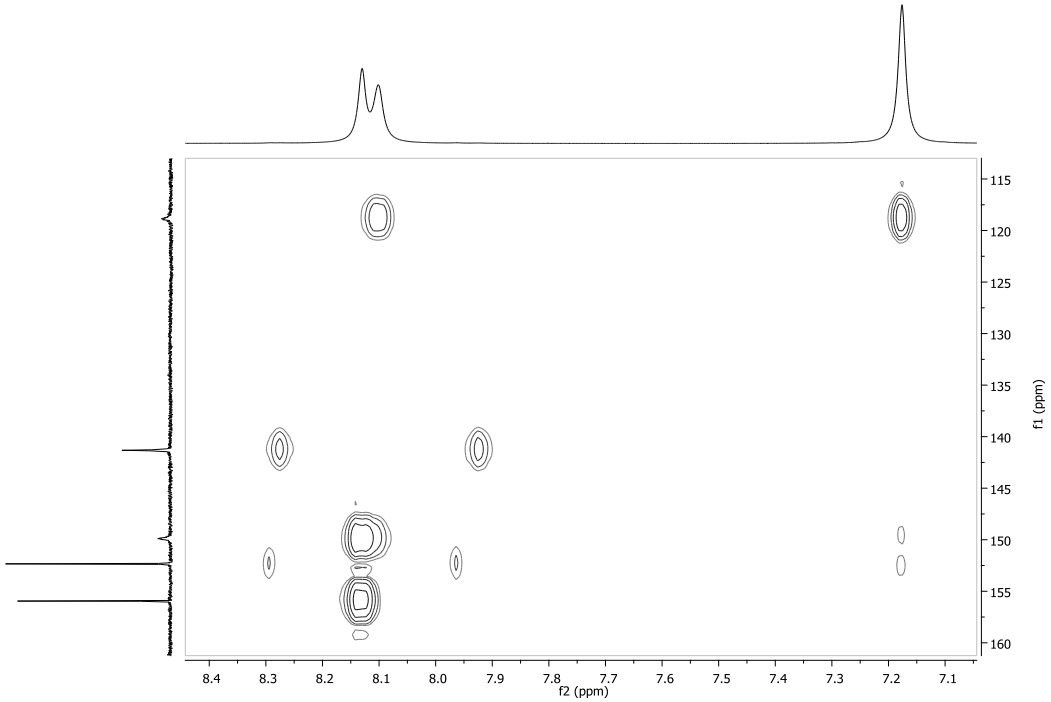
Spectrum 38. ^{13}C NMR of 9-(Cyclohexylmethyl)-*9H*-purin-6-amine (**7c**).



Spectrum 39. HSQC of 9-(Cyclohexylmethyl)-*9H*-purin-6-amine (**7c**).

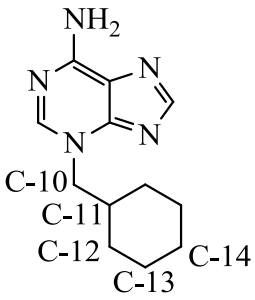


Spectrum 40. HMBC of 9-(Cyclohexylmethyl)-*9H*-purin-6-amine (**7c**).



Spectrum 41. HMBC of 9-(Cyclohexylmethyl)-*9H*-purin-6-amine (**7c**), expansion of the aromatic region.

3-(Cyclohexylmethyl)-*3H*-purin-6-amine (28c)



28c

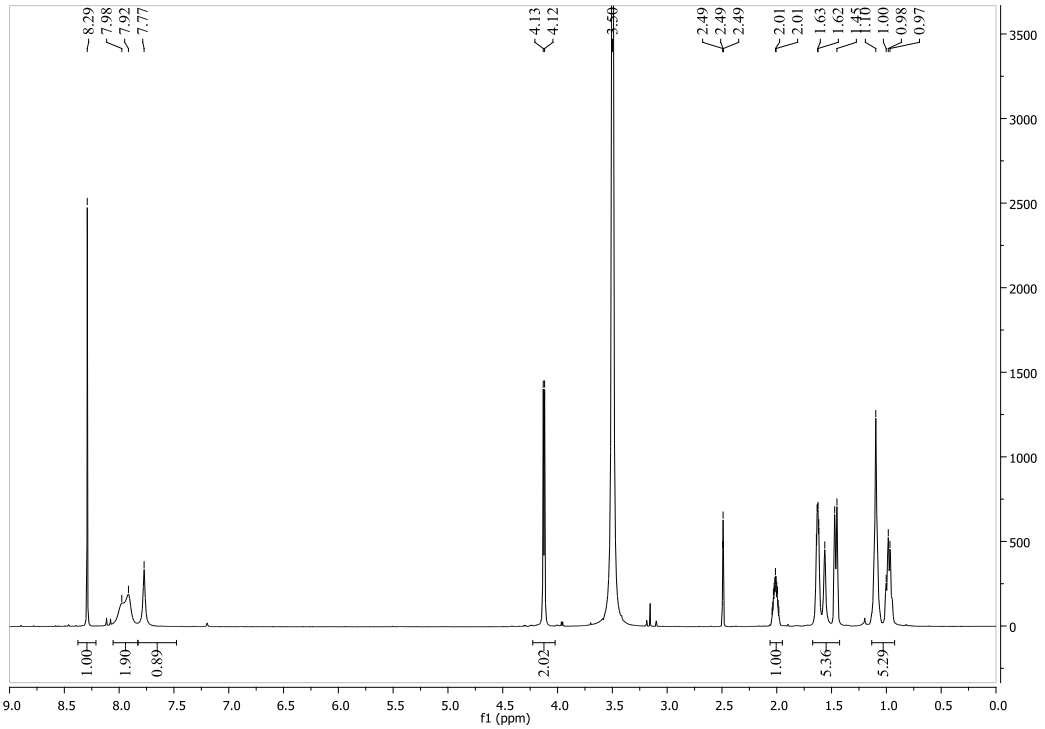
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.29 (s, 1H, H-2), 7.98 and 7.92 (br d, 2H, NH_2), 7.77 (s, 1H, H-8), 4.12 (d, J = 7.4 Hz, 2H, H-10), 2.01 (dtt, J = 15.0, 7.5, 3.8 Hz, H-11), 1.63 – 1.45 (m, 5H, H-12, H-13 and H-14), 1.10 – 0.97 (m, 5H, H-12, H-13 and H-14).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 155.0 (C-6), 152.3 (C-2), 149.8 (C-4), 143.9 (C-8), 120.2 (C-5), 55.1 (C-10), 36.4 (C-11), 29.8 (C-12), 25.9 (C-14), 25.1 (C-13).

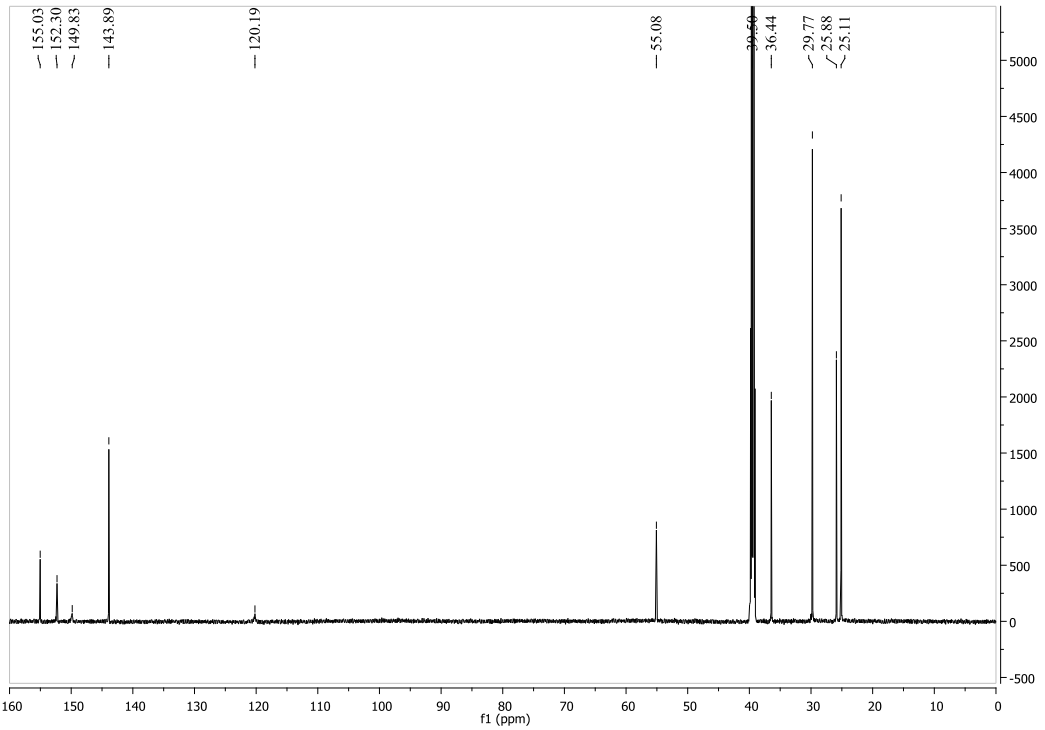
MS EI *m/z* (rel. %) 231 (72, M^+), 188 (12), 149 (100), 148 (76), 136 (22), 135 (80), 108 (15).

HR-MS Found 231.1479, calculated for $C_{12}H_{17}N_5$ 231.1484.

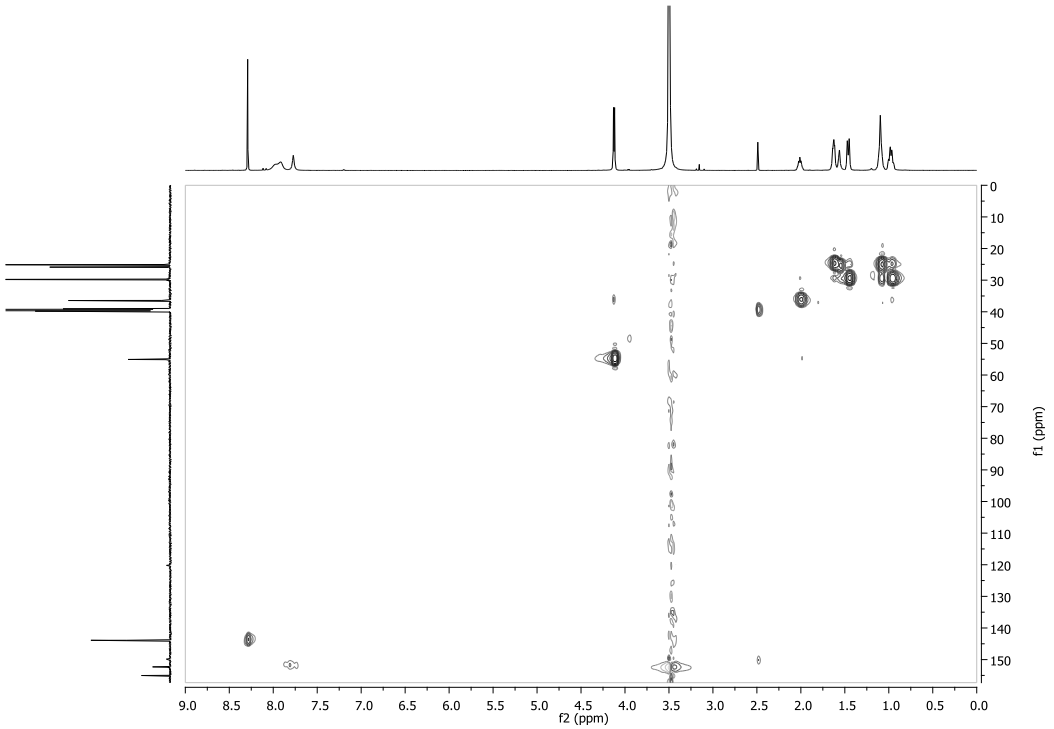
M.p. 250-252 °C.



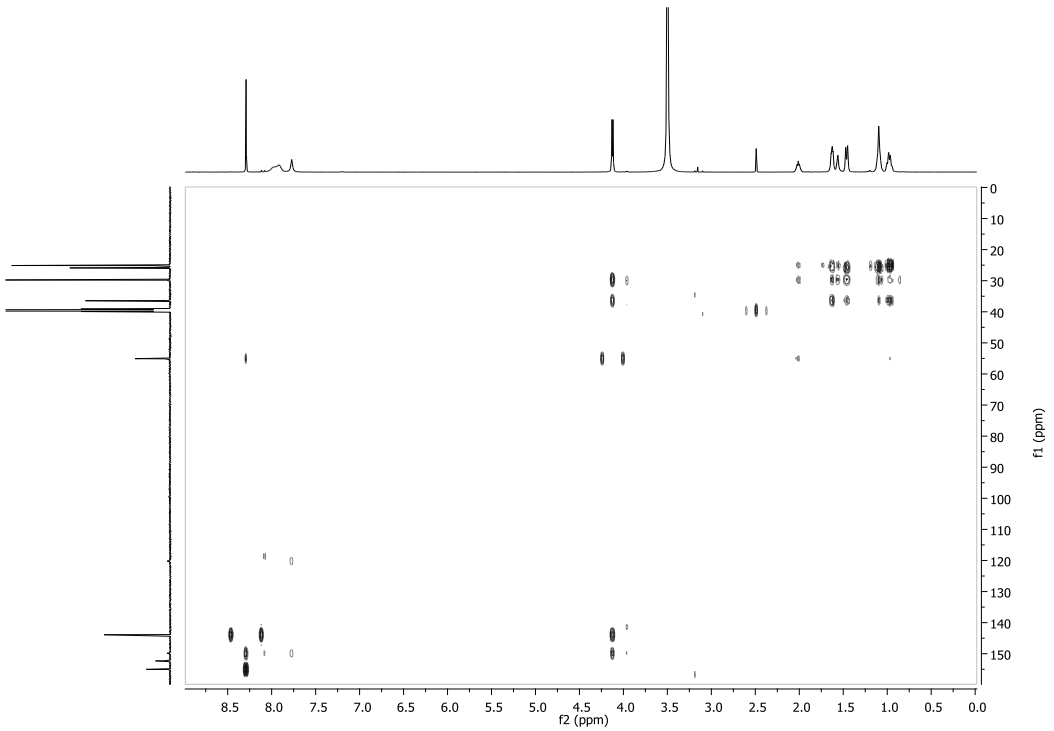
Spectrum 42. ^{1}H NMR of 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (**28c**).



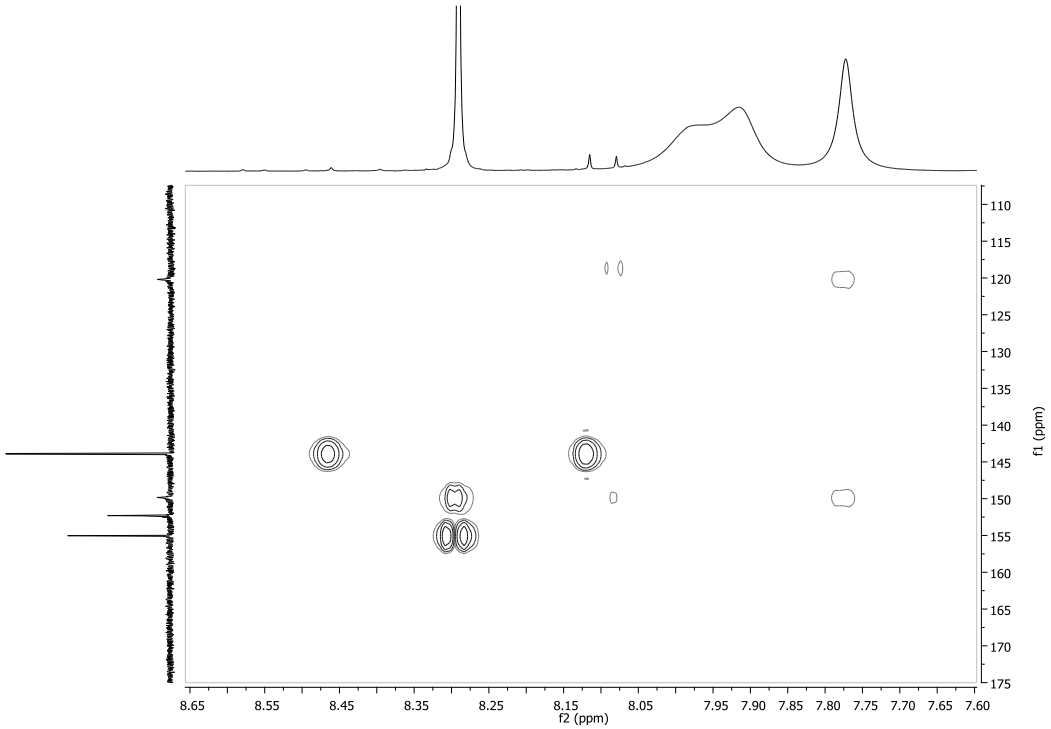
Spectrum 43. ^{13}C NMR of 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (**28c**).



Spectrum 44. HSQC of 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (**28c**).

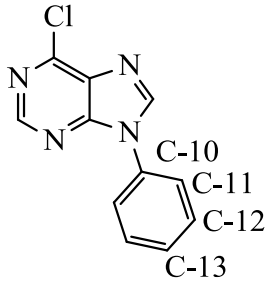


Spectrum 45. HMBC of 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (**28c**).



Spectrum 46. HMBC of 3-(Cyclohexylmethyl)-*3H*-purin-6-amine (**28c**), expansion of the aromatic region.

6-Chloro-9-phenyl-*9H*-purine (14)



14

6-Chloropurine (**13**) (152 mg, 1.05 mmol), phenylboronic acid (371 mg, 3.04 mmol), 1,10-phenanthroline (366 mg, 2.03 mmol), anhydrous copper(II) acetate (188 mg, 1.04 mmol) and 982 mg 3Å molecular sieves were weighed into a flask. Dry DCM (15 mL) was added, a condenser attached and the reaction mixture stirred at ambient temperature in open atmosphere for 4 days. Methanol (150 mL) was added and the mixture was filtered through Celite, evaporated and purified by flash chromatography (1:3 ethylacetate:hexanes) to give **14** as a white powder (147 mg, 72%).

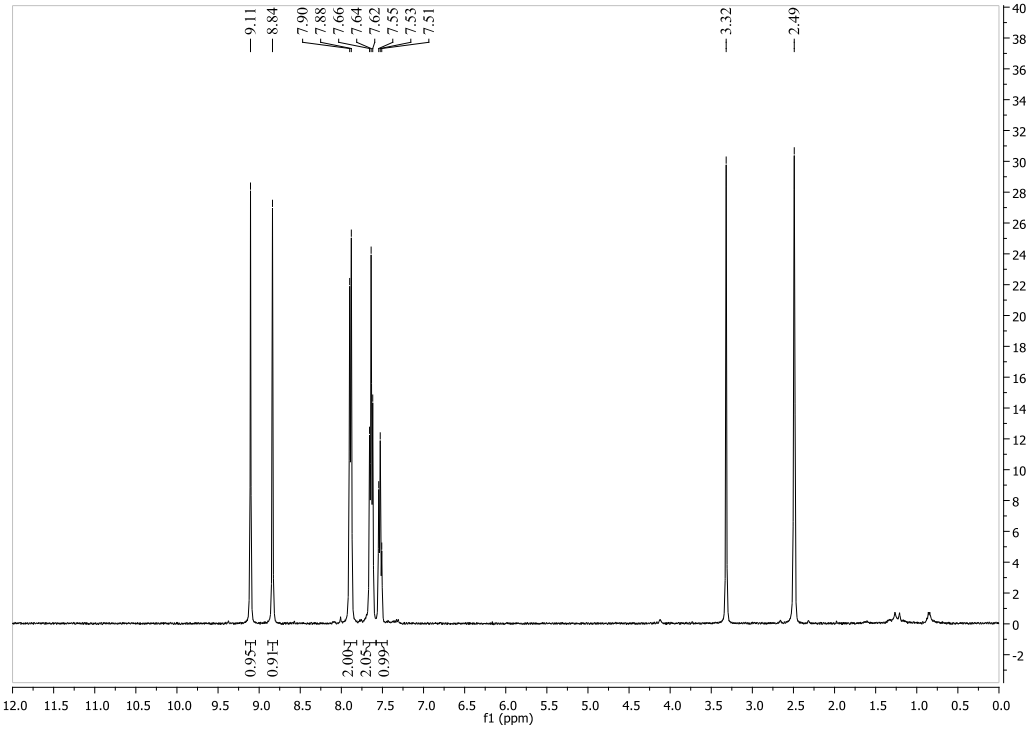
^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.11 (s, 1H, H-8), 8.84 (s, 1H, H-2), 7.89 (d, J = 7.9 Hz, 2H, H-11), 7.64 (t, J = 7.7 Hz, 2H, H-12), 7.53 (t, J = 7.3 Hz, 1H, H-13).

^{13}C NMR (DMSO-d_6, 100 MHz) δ 152.2 (C-2), 151.5 (C-4), 149.6 (C-6), 146.4 (C-8), 134.0 (C-10), 131.5 (C-5), 129.6 (C-12), 128.5 (C-13), 123.7 (C-11).

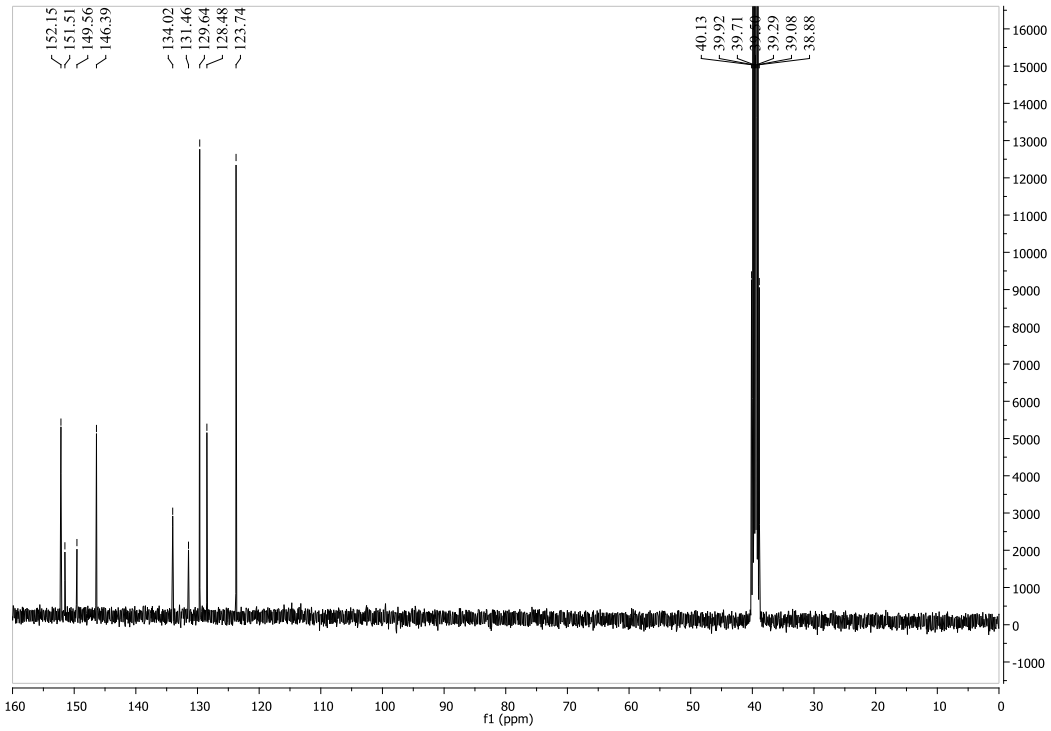
MS EI *m/z* (rel. %) 232/230 (32/100, M^+), 195 (14), 168 (12), 141 (7), 77 (16).

HR-MS Found 230.0356, calculated for $C_{11}H_7ClN_4$ 230.0359.

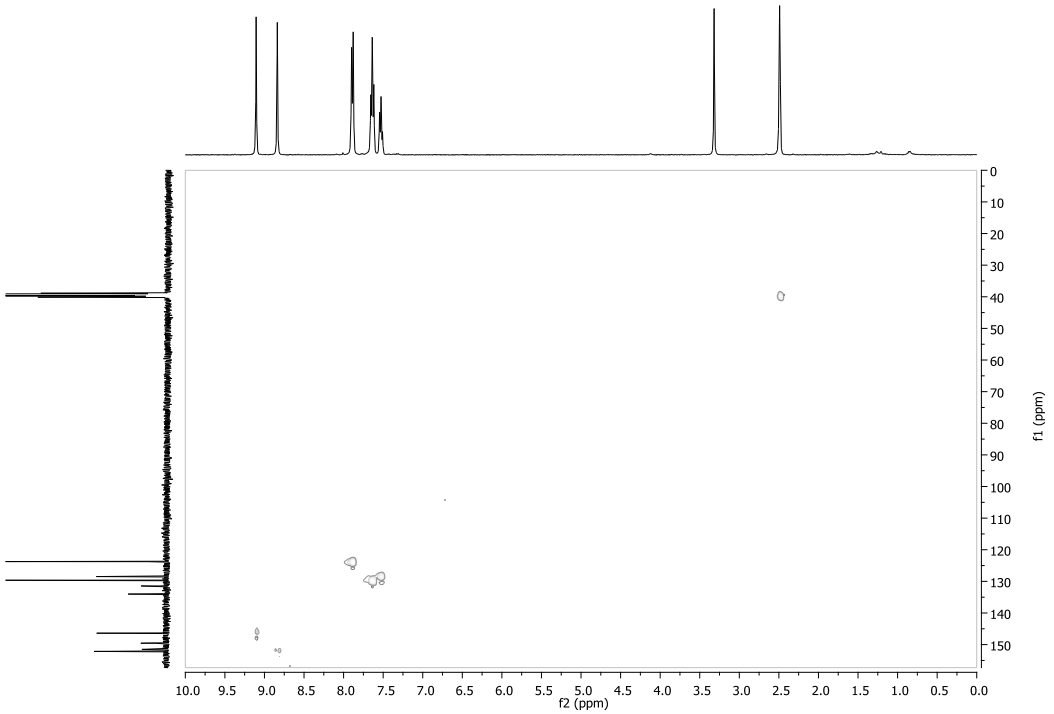
M.p. 250-252 °C.



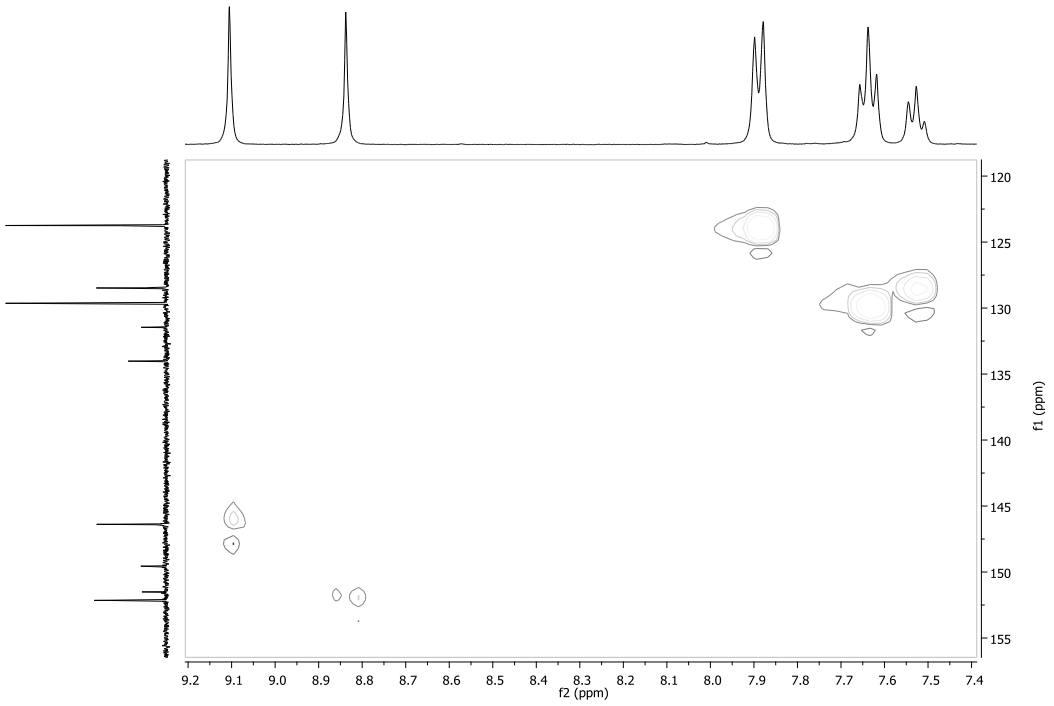
Spectrum 47. ^{1}H NMR of 6-Chloro-9-phenyl-*9H*-purine (**14**).



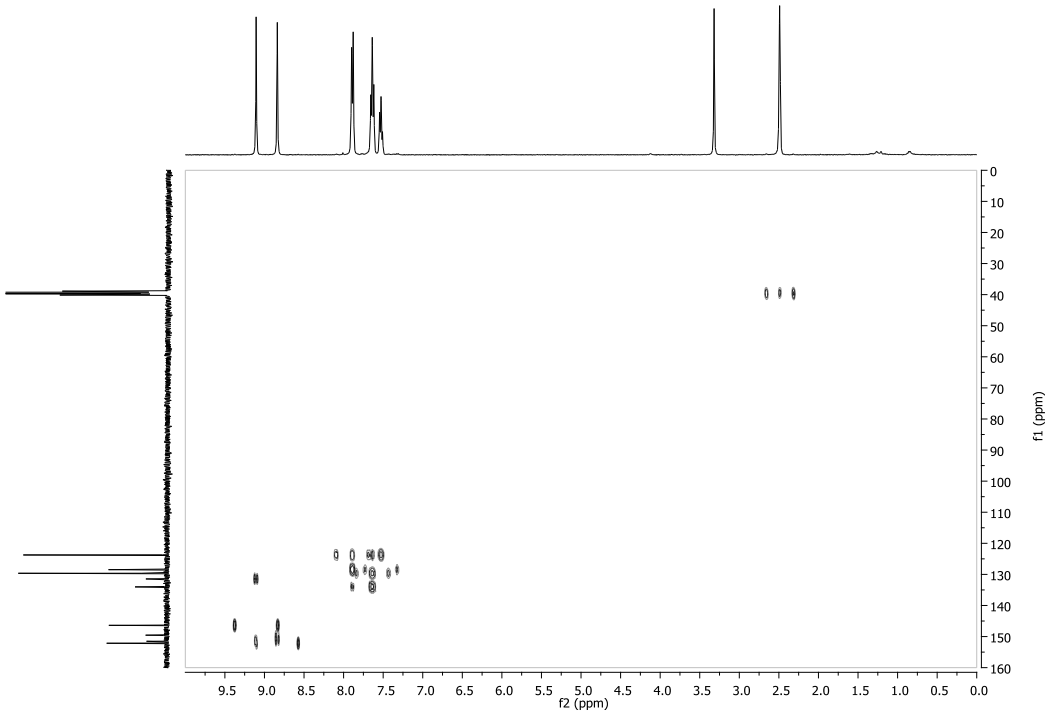
Spectrum 48. ^{13}C NMR of 6-Chloro-9-phenyl-*9H*-purine (**14**).



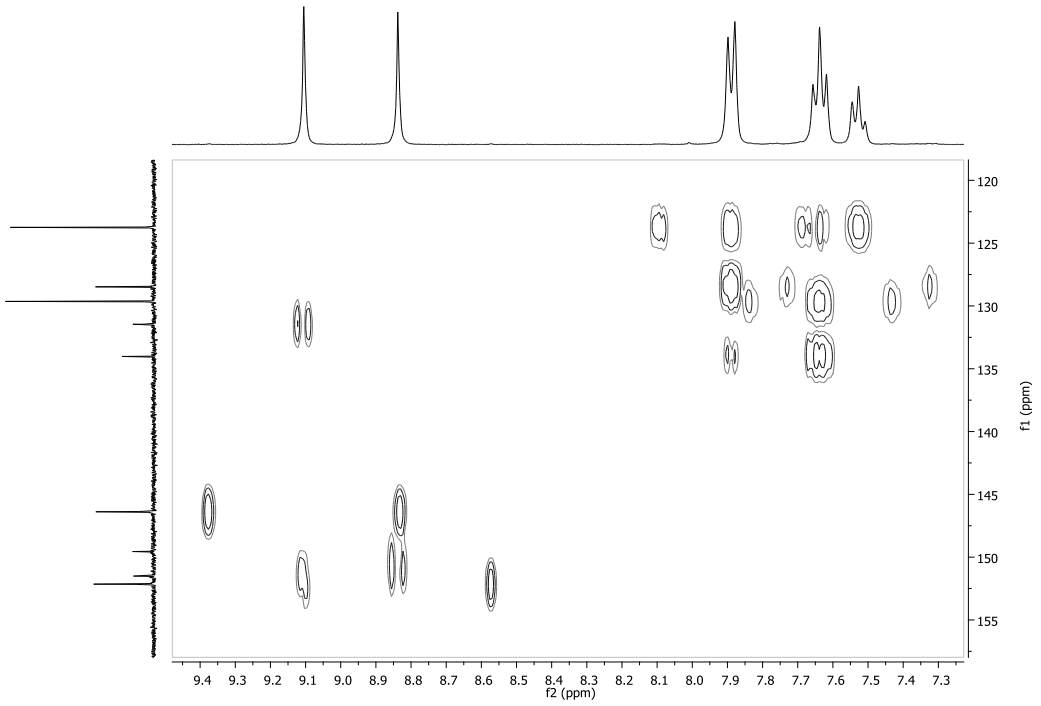
Spectrum 49. HSQC of 6-Chloro-9-phenyl-*9H*-purine (**14**).



Spectrum 50. HSQC of 6-Chloro-9-phenyl-*9H*-purine (**14**), expansion of the aromatic region.

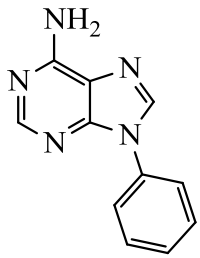


Spectrum 51. HMBC of 6-Chloro-9-phenyl-*9H*-purine (**14**).

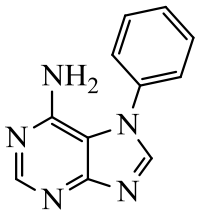


Spectrum 52. HMBC of 6-Chloro-9-phenyl-*9H*-purine (**14**), expansion of the aromatic region.

9-Phenyl-*9H*-purin-6-amine (7d) and 7-Phenyl-*7H*-purin-6-amine (29d)



7d



29d

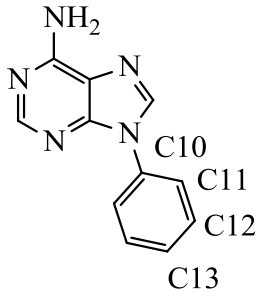
Method 1 (from adenine): A mixture of adenine (**4**) (70 mg, 0.52 mmol), phenylboronic acid (136 mg, 1.12 mmol), copper(II) acetate monohydrate (101 mg, 0.506 mmol) and TMEDA (0.15 mL, 1.0 mmol) was stirred vigorously in a mixture of 10 mL distilled water and 40 mL methanol for 21.5 h at ambient temperature. The solvent was evaporated *in vacuo* and the residue purified by column chromatography (2.5-10% methanol in dichloromethane) to give **7d** as a colourless powder (22 mg, 20%).

Method 2 (from adenine): A mixture of adenine (**4**) (135 mg, 1.00 mmol), phenyl boronic acid (184 mg, 1.51 mmol), 1,10-phenanthroline (270 mg, 1.50 mmol) and copper acetate monohydrate (303 mg, 1.52 mmol) was dissolved in dry DMF (5 mL) and a condenser attached. The reaction was stirred at 90 °C for 24 h, and then the solvent was evaporated *in vacuo*. The residue was purified as for Method 1 to give **7d** as a colourless powder (44 mg, 21%).

Method 3 (from adenine): A mixture of adenine (**4**) (136.0 mg, 1.007 mmol), phenyl boronic acid (184.2 mg, 1.510 mmol), 1,10-phenanthroline (270.9 mg, 1.505 mmol) and anhydrous copper acetate (277.1 mg, 1.528 mmol) was dissolved in dry DMF (5 mL) and a condenser attached. The reaction was stirred at 90 °C for 24 h, and then the solvent was evaporated *in vacuo*. The residue was purified as for Method 1 to give **7d** as a colourless powder (37 mg, 17%) and **29d** as an off-white powder (54 mg, small impurities, ~25%).

Method 4 (from 6-chloro-9-phenylpurine): 6-Chloro-9-phenylpurine (**14**) (115 mg, 0.499 mmol) was dissolved in 5 mL concentrated ammonium hydroxide and heated at 100 °C in a sealed vial overnight. The ammonium hydroxide was evaporated *in vacuo* and the residue purified using flash chromatography (2-5% methanol in dichloromethane) to give **7d** as a colourless powder (100 mg, 95%).

9-Phenyl-*9H*-purin-6-amine (7d)



7d

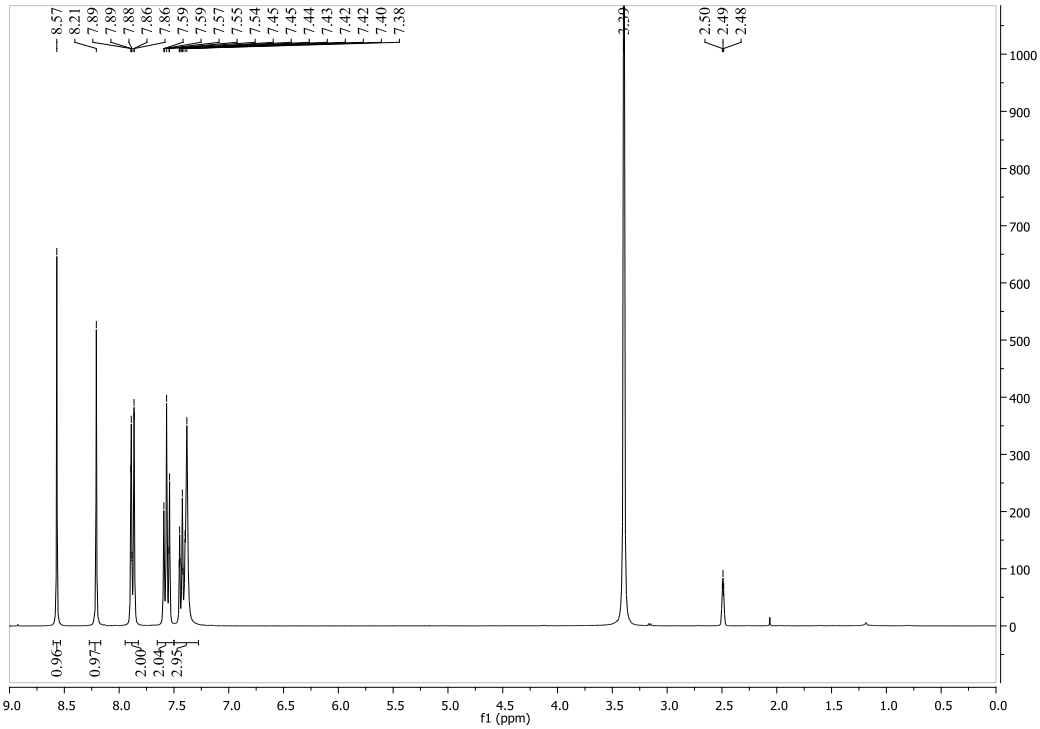
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.57 (s, 1H, H-2), 8.21 (s, 1H, H-8), 7.89 – 7.86 (m, 2H, H-11), 7.59 – 7.54 (m, 2H, H-12), 7.43 – 7.40 (m, 1H, H-13), 7.38 (br s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.4 (C-6), 153.2 (C-2), 149.2 (C-4), 139.7 (C-7), 135.1 (C-10), 129.5 (C-12), 127.4 (C-13), 123.0 (C-11), 119.3 (C-5).

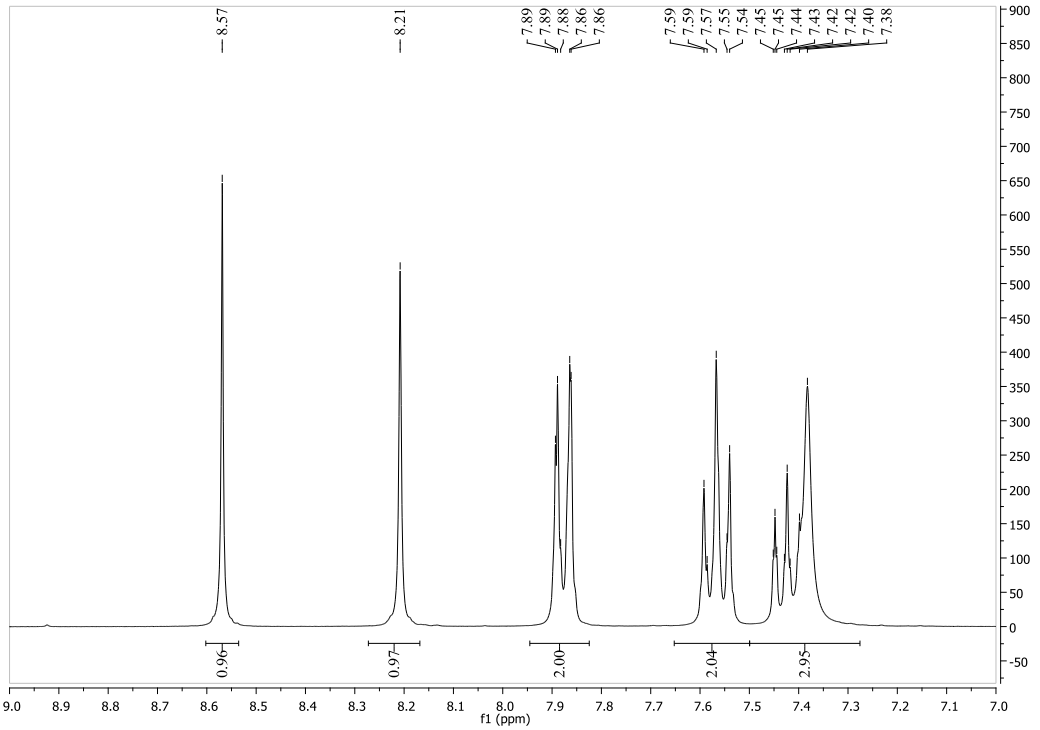
MS EI *m/z* (rel. %) 211 (100, M^+), 184 (13), 104 (7), 77 (13).

HR-MS Found 211.0855, calculated for $C_{11}H_9N_5$ 211.0858.

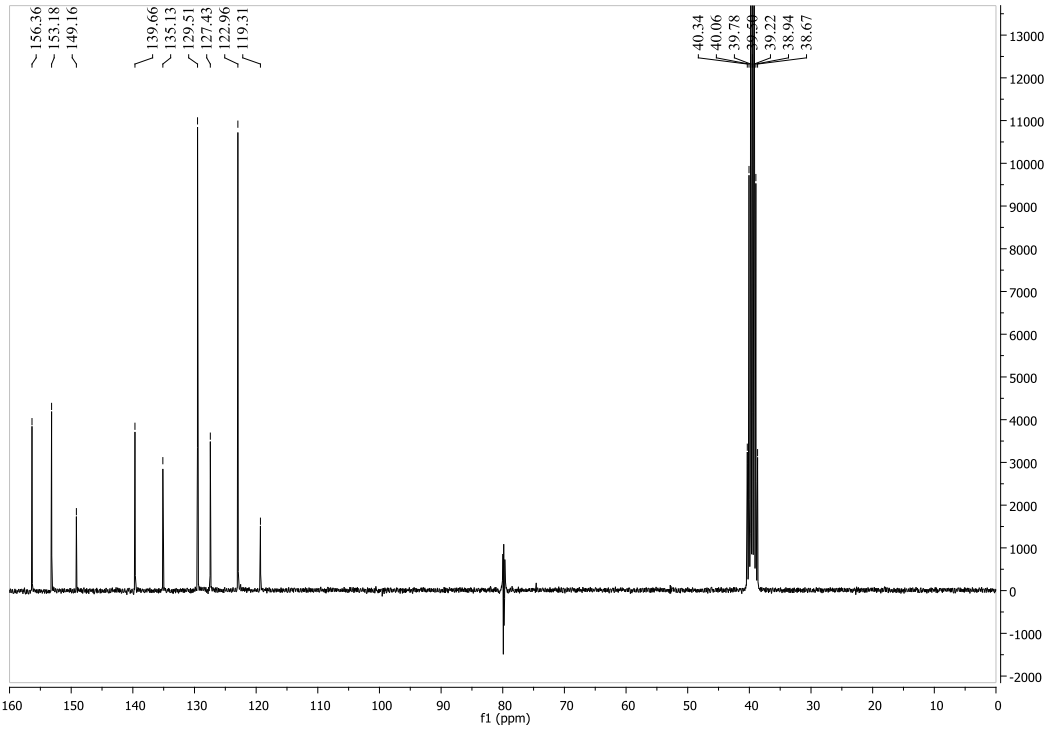
M.p. 244-246 °C (lit.[94] = 245-6 °C).



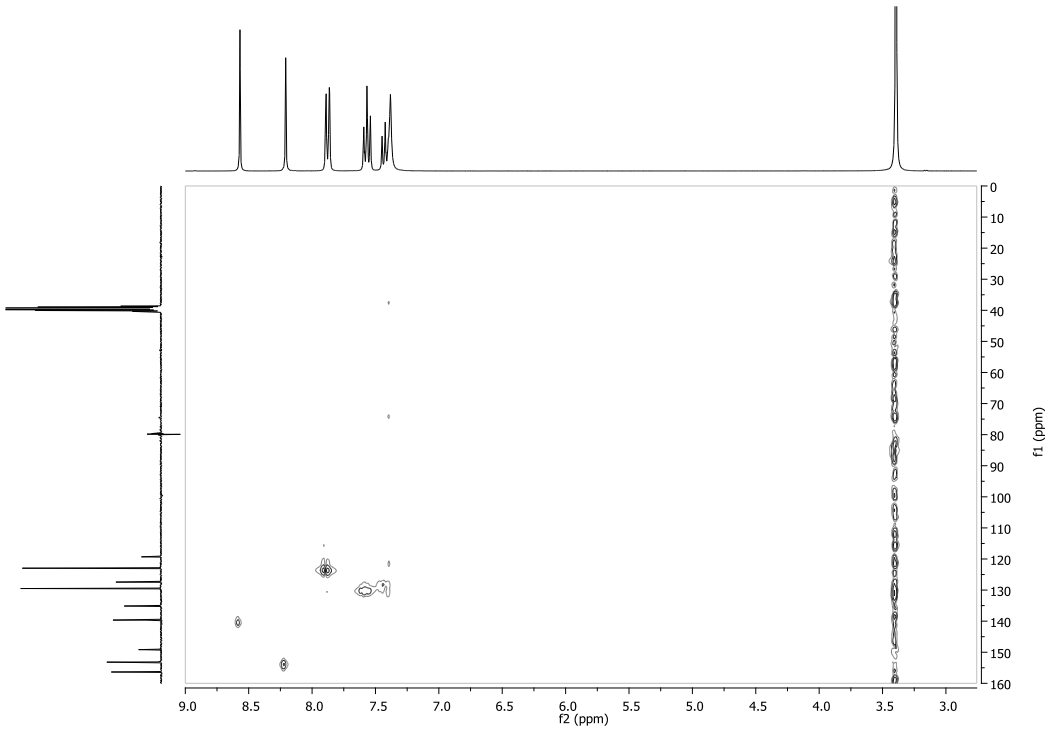
Spectrum 53. ^{1}H NMR of 9-Phenyl-*9H*-purin-6-amine (**7d**).



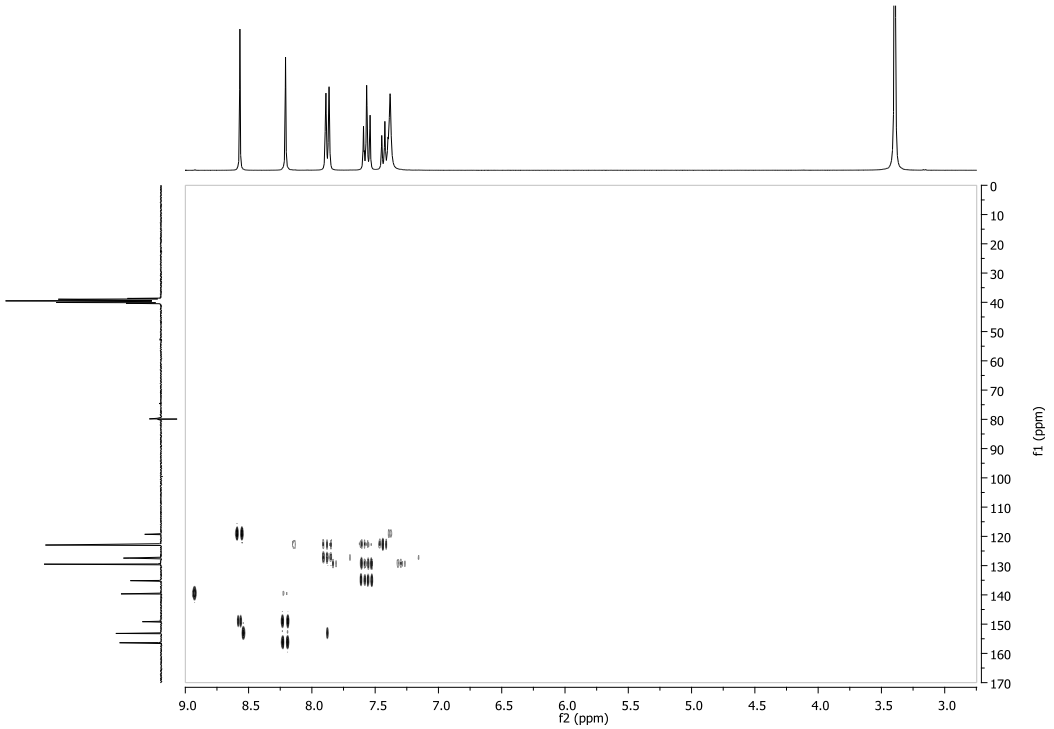
Spectrum 54. ^{1}H NMR of 9-Phenyl-*9H*-purin-6-amine (**7d**), expansion of the aromatic region.



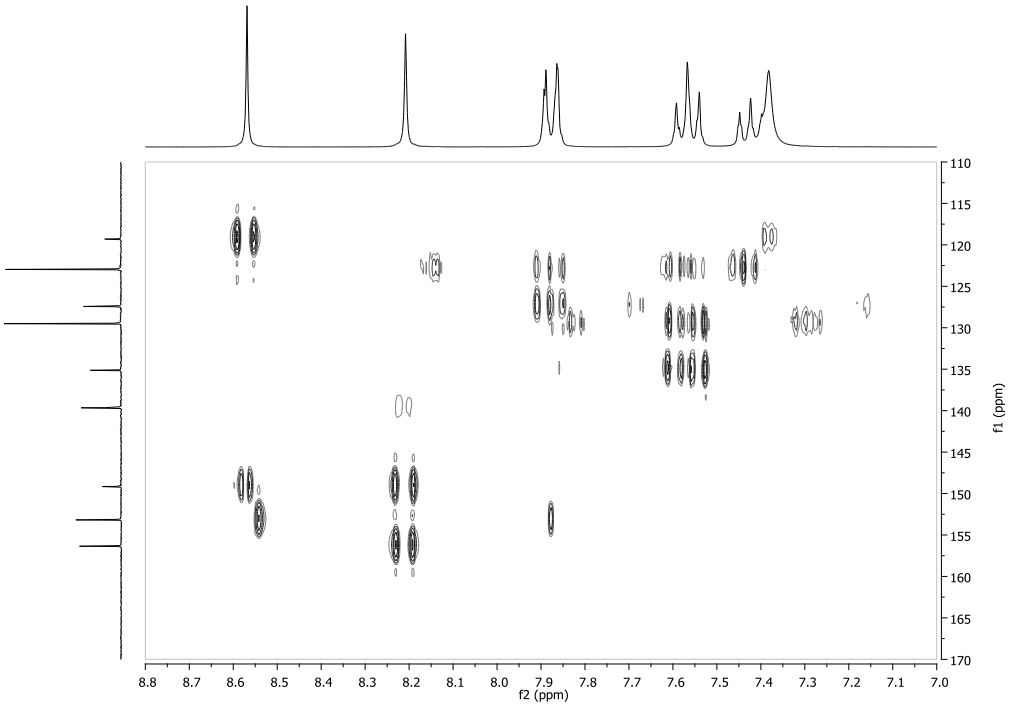
Spectrum 55. ^{13}C NMR of 9-Phenyl-*9H*-purin-6-amine (**7d**).



Spectrum 56. HMQC of 9-Phenyl-*9H*-purin-6-amine (**7d**).

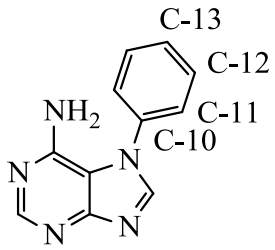


Spectrum 57. HMBC of 9-Phenyl-*9H*-purin-6-amine (**7d**).



Spectrum 58. HMBC of 9-Phenyl-*9H*-purin-6-amine (**7d**), expansion of the aromatic region.

7-Phenyl-*7H*-purin-6-amine (29d)



29d

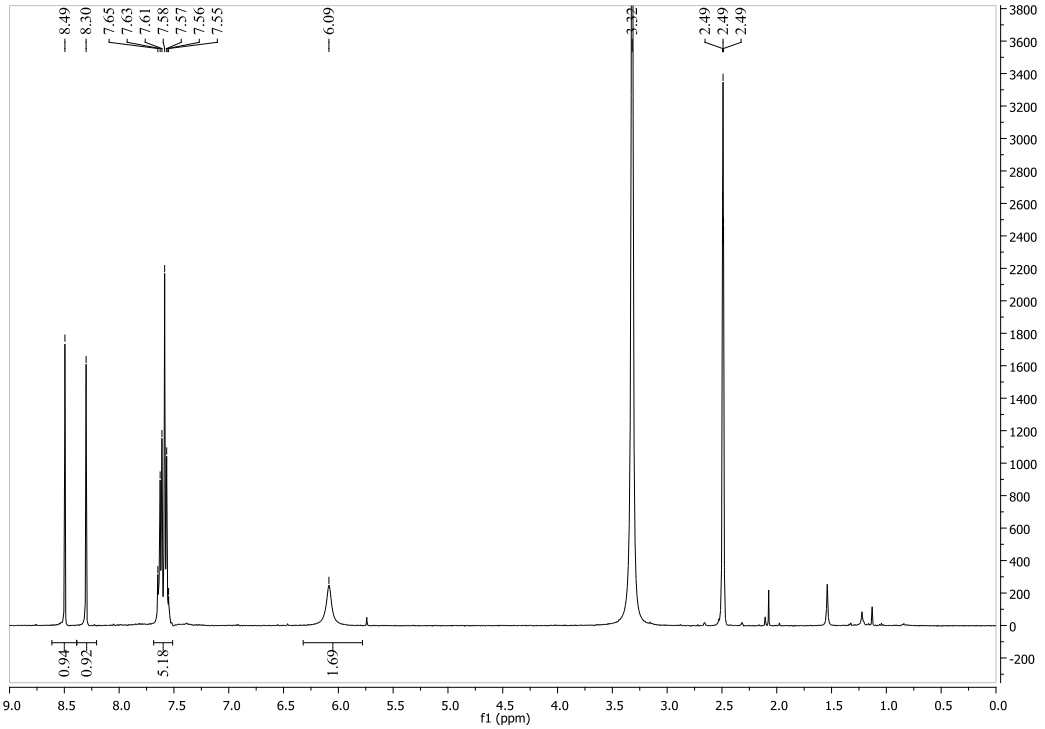
^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.49 (s, 1H, H-8), 8.30 (s, 1H, H-2), 7.68 – 7.51 (m, 5H, H-11, H-12 and H-13), 6.09 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.9 (C-4), 152.7 (C-2), 151.1, 145.8 (C-8), 135.4 (C-10), 129.9 (C-11 or C-12), 129.0 (C-13), 125.6 (C-11 or C-12), 110.5 (C-5).

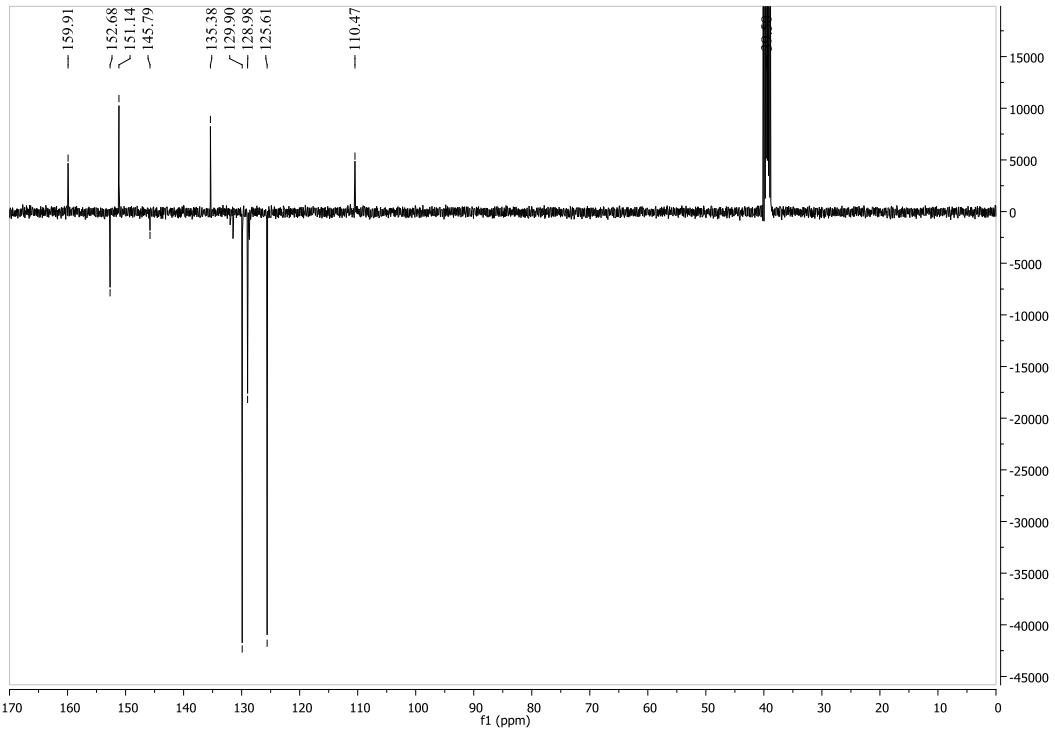
MS EI *m/z* (rel. %) 211 (100, M^+), 157 (11), 104 (14), 77 (23).

HR-MS Found 211.085116, calculated for $C_{11}H_9N_5$ 211.0858.

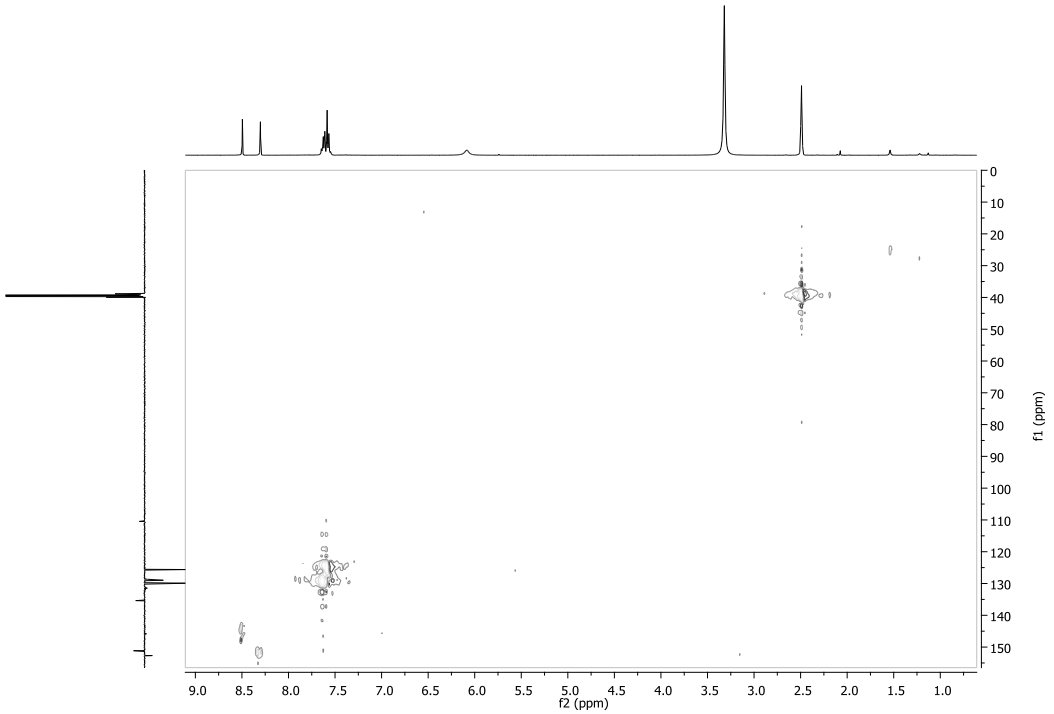
M.p. not obtained.



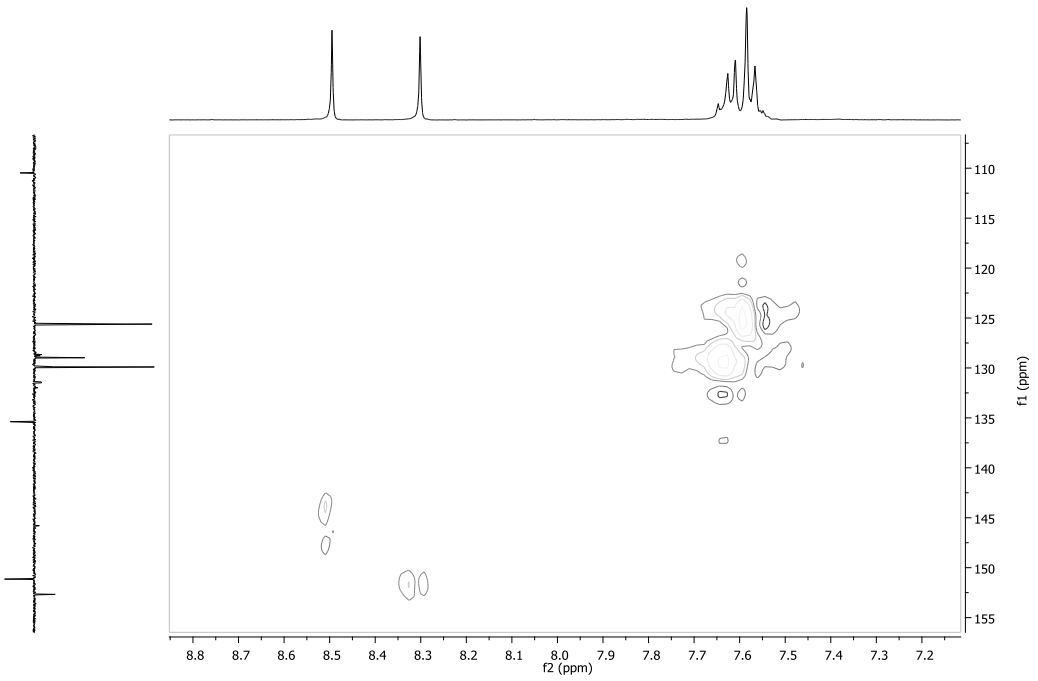
Spectrum 59. ^{1}H NMR of 7-Phenyl-*7H*-purin-6-amine (**29d**).



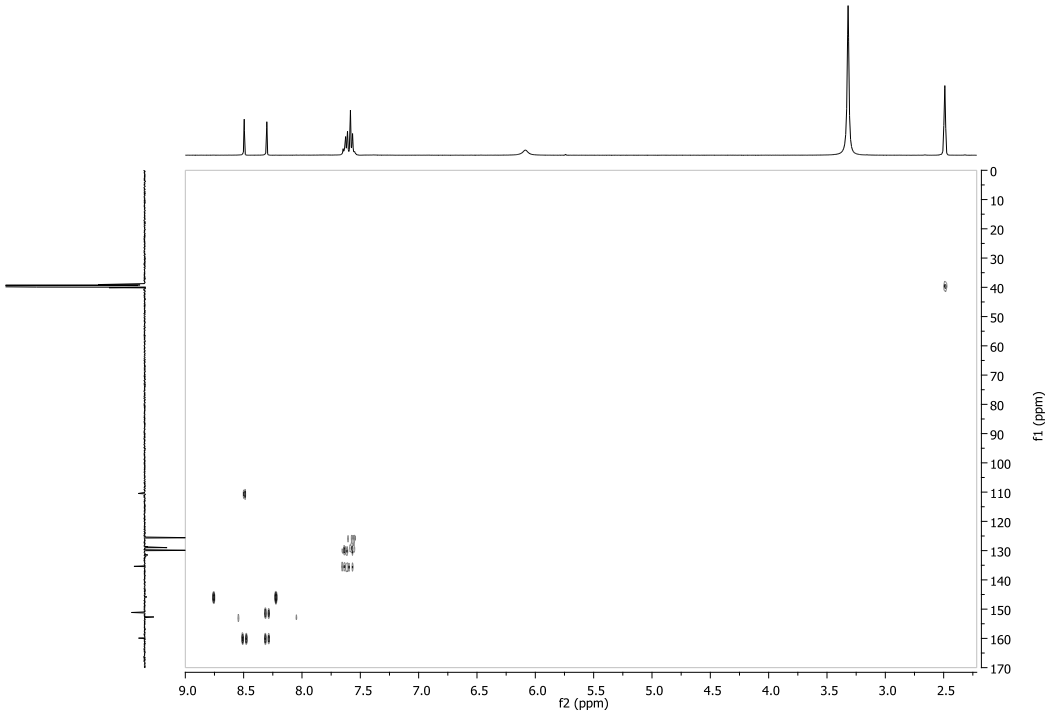
Spectrum 60. ^{13}C NMR of 7-Phenyl-*7H*-purin-6-amine (**29d**).



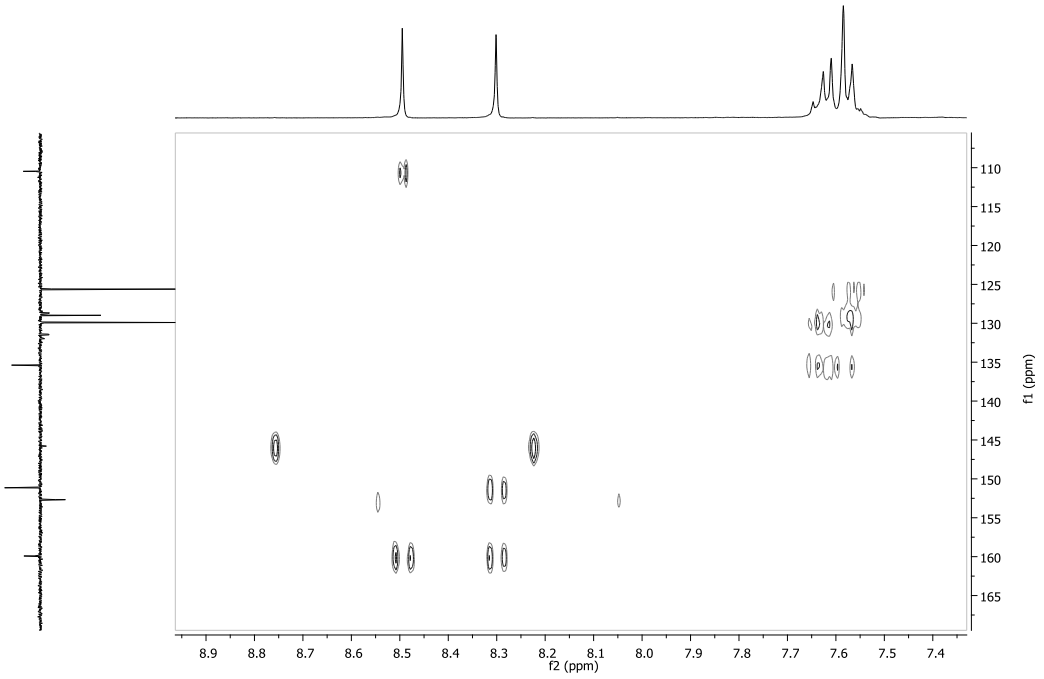
Spectrum 61. HSQC of 7-Phenyl-*7H*-purin-6-amine (**29d**).



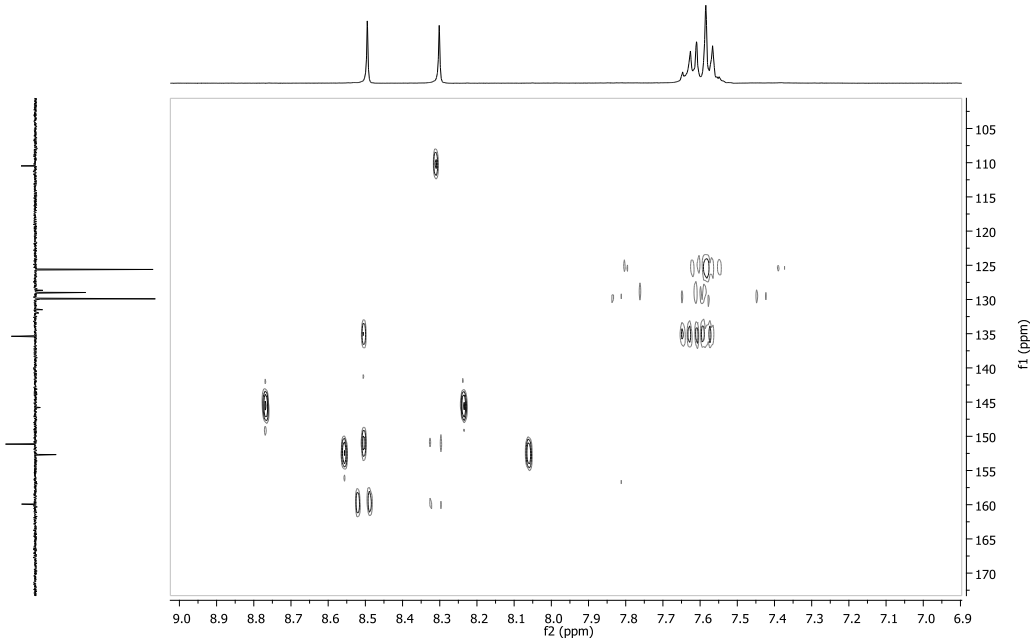
Spectrum 62. HSQC of 7-Phenyl-*7H*-purin-6-amine (**29d**), expansion of the aromatic region.



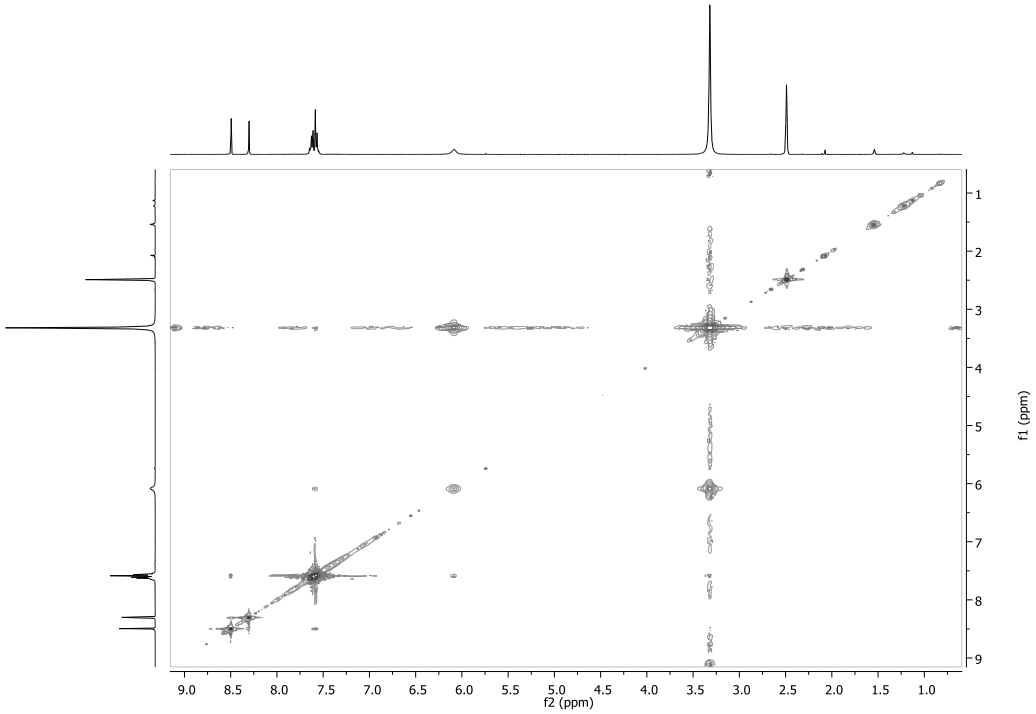
Spectrum 63. HMBC of 7-Phenyl-*7H*-purin-6-amine (**29d**).



Spectrum 64. HMBC of 7-Phenyl-*7H*-purin-6-amine (**29d**), expansion of the aromatic region.

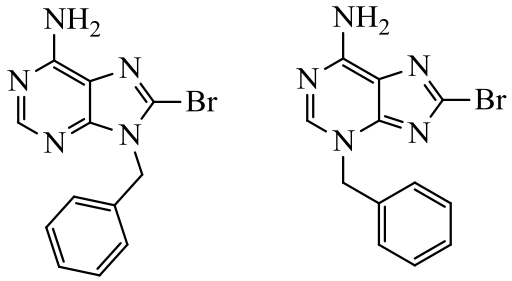


Spectrum 65. HMBC of 7-Phenyl-*7H*-purin-6-amine (**29d**), expansion of the aromatic region showing the coupling of H-8 to C-10. N.b. Long range ^{1}H-C *J* coupling constant = 2 Hz.



Spectrum 66. NOESY of 7-Phenyl-*7H*-purin-6-amine (**29d**).

9-Benzyl-8-bromo-*9H*-purin-6-amine (6a) and 3-Benzyl-8-bromo-*3H*-purin-6-amine (30a)



6a **30a**

Method 1: A mixture of 8-bromoadenine (**5**) (126 mg, 1.03 mmol), DMF (5 mL) and potassium carbonate (280 mg, 2.03 mmol) was stirred at ambient temperature under a nitrogen atmosphere for 30 minutes. Benzyl bromide (0.18 mL, 1.5 mmol) was added and the mixture stirred for another 4 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (0-5% methanol in dichloromethane) to give compounds **6a** (45 mg, 15%) and **30a** (128 mg, 42%) as colourless powders.

Method 2: A mixture of 9-benzyladenine (**7a**) (193 mg, 0.857 mmol) and *N*-bromosuccinimide (497 mg, 2.79 mmol) was weighed into a flask and the system flushed with nitrogen gas. Dry DMF (4 mL) was added and stirred at ambient temperature for 24 h. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography (1:1 ethyl acetate: hexanes – ethyl acetate) to give **6a** as a pale reddish powder (121 mg, 42%).

Method 3: A mixture of 9-benzyladenine (**7a**) (147 mg, 0.653 mmol) and *N*-bromosuccinimide (601 mg, 3.38 mmol) was weighed into a flask and the system flushed with nitrogen gas. Chloroform (10 mL) was added and refluxed for 8 h. The crude mixture was washed with 2 x 10 mL saturated sodium sulfite and 2 x 10 mL saturated sodium chloride solutions. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography (1:1 ethyl acetate:hexanes – ethyl acetate – 5-10% methanol in dichloromethane) to give **6a** as a colourless powder (32 mg, 16%).

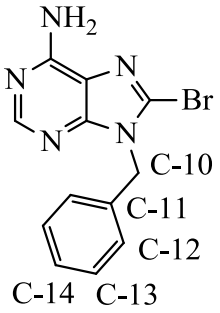
7.1.1. X-ray Crystallographic Analysis of 30a

Crystals of 3 suitable for X-ray crystallography were obtained from a solution of compound **30a** in acetonitrile placed inside a larger vial containing ethyl acetate. They are unstable at room temperature due to loss of co-crystallized ethyl acetate solvent molecules, and X-ray data collection with Apex-2[95] was thus performed at 105 K. Apex II single crystal CCD-diffractometer, MoK_α radiation (λ = 0.71069 Å), 0.30 x 0.30 x 0.26 mm block-shaped specimen, data integration and cell refinement with SAINT-Plus,[96] absorption correction by SADABS,[97] structure solution by and least-squares refinement on F^2 with SHELXTL.[98]

Solvent molecules are located on two different inversion centres, each with a maximum allowed occupancy of 0.500 and form distinct channels running through the crystal along the *ab*-diagonal. The geometries of independent solvent molecules were constrained to be similar within a standard deviation of 0.002 Å for bond lengths and 0.003 Å for 1-3 distances.

3-Benzyl-8-bromo-*3H*-purin-6-amine ethyl acetate solvate: $C_{12}H_{10}BrN_5 \cdot 0.5C_4H_8O_2$, M = 348.21, triclinic, *P*-1, *a* = 8.4937(6) Å, *b* = 12.9096(9) Å, *c* = 15.2238(10) Å, α = 101.723(1)°, β = 105.162(1)°, γ = 108.192(1)°, *Z* = 4, $N_{observed}$ = 5830, $R[F^2 > 2\sigma(F^2)]$ = 0.058, $wR(F^2)$ = 0.146, CCDC 786213.

9-Benzyl-8-bromo-*9H*-purin-6-amine (6a)



6a

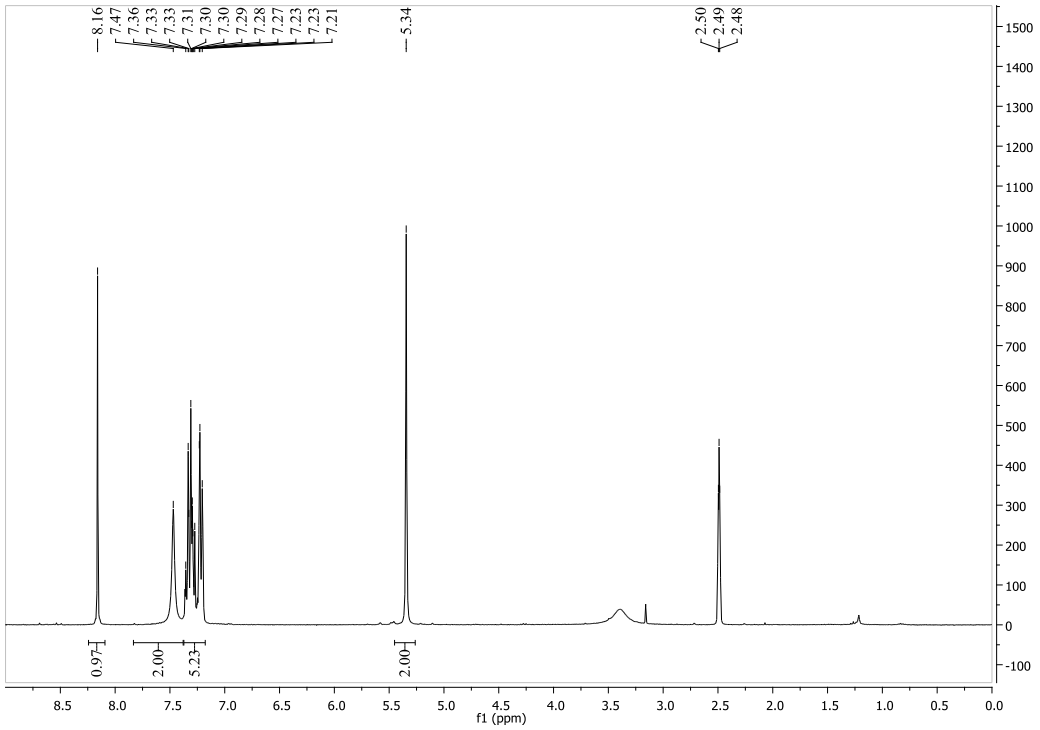
^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (s, 1H, H-2), 7.47 (br s, 2H, NH_2), 7.33 – 7.21 (m, 5H, H-12, H-13 and H-14), 5.35 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.7 (C-6), 153.0 (C-2), 150.9 (C-4), 135.9 (C-8), 128.7 (C-12 or C-13), 127.8 (C-14), 127.1 (C-12 or C-13), 126.5 (C-11), 119.0 (C-5), 46.6 (C-10).

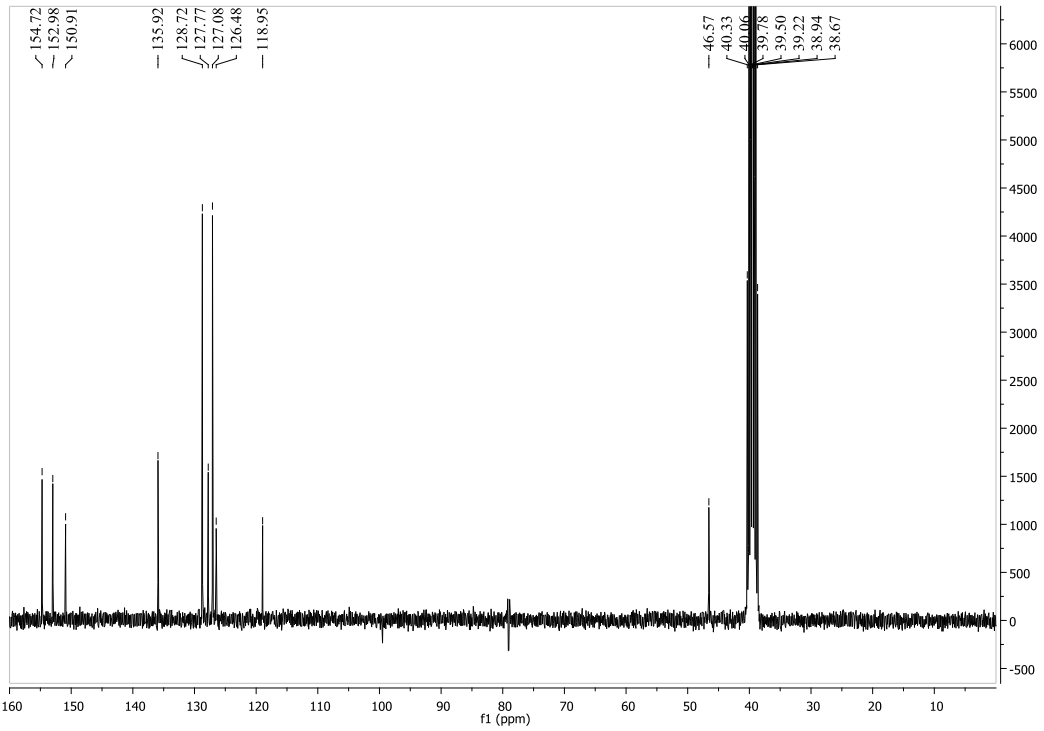
MS EI *m/z* (rel. %) 305/303 (26/26, M^+), 304/302 (19/15), 224 (52), 91 (100).

HR-MS Found 303.0112, calculated for $C_{12}H_{10}BrN_5$ 303.0120.

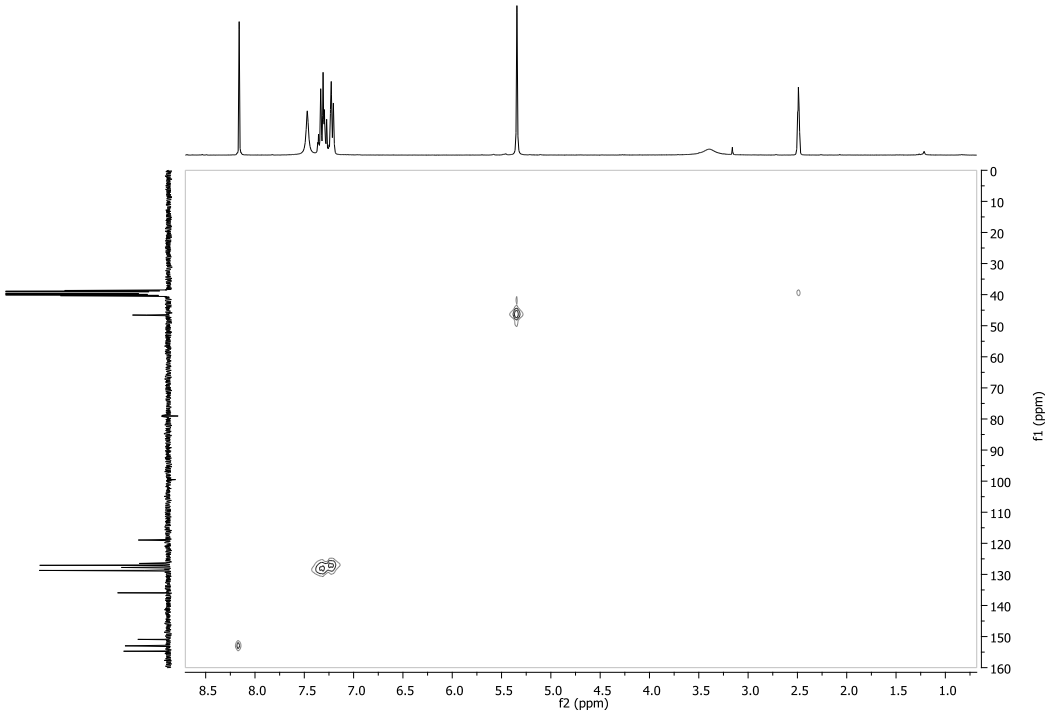
M.p. 238 °C (lit.[34] = 227-230 °C).



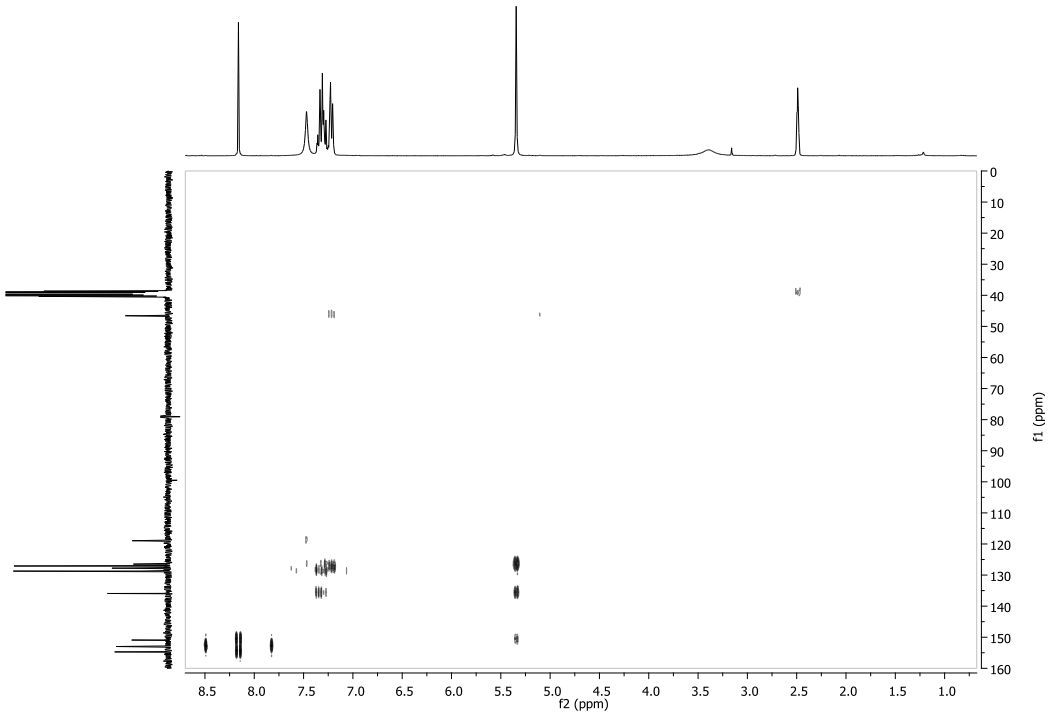
Spectrum 67. ^{1}H NMR of 9-Benzyl-8-bromo-*9H*-purin-6-amine (**6a**).



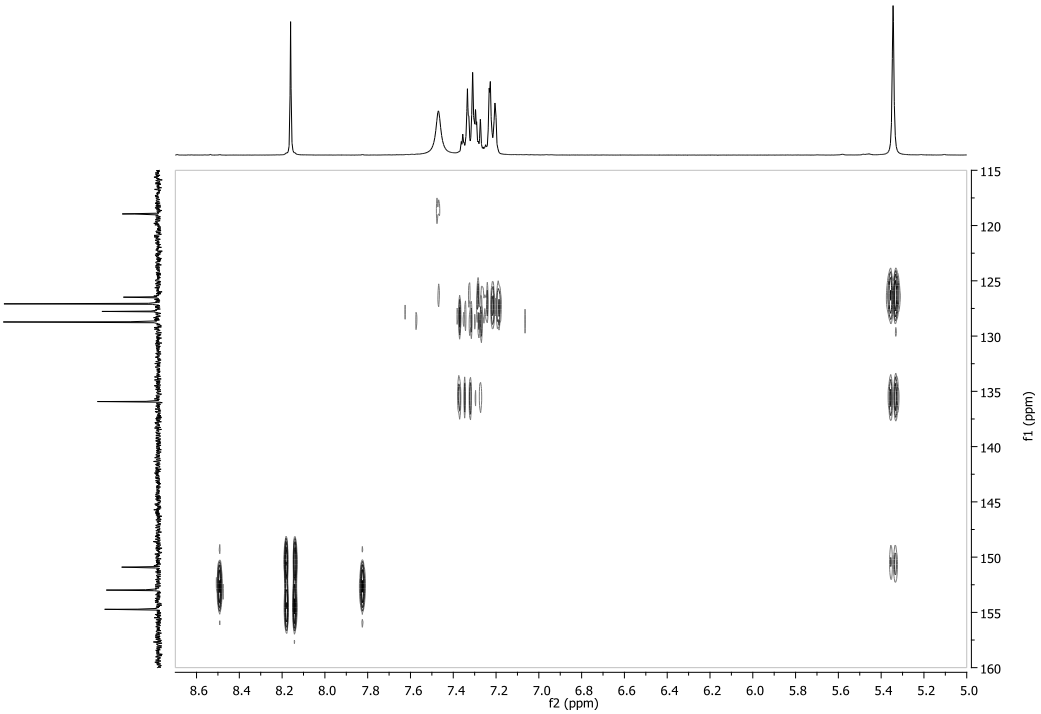
Spectrum 68. ^{13}C NMR of 9-Benzyl-8-bromo-*9H*-purin-6-amine (**6a**).



Spectrum 69. HMQC of 9-Benzyl-8-bromo-*9H*-purin-6-amine (**6a**).

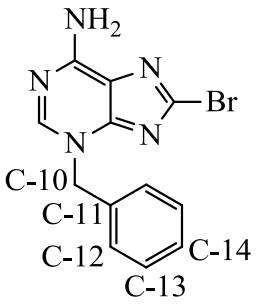


Spectrum 70. HMBC of 9-Benzyl-8-bromo-*9H*-purin-6-amine (**6a**).



Spectrum 71. HMBC of 9-benzyl-8-bromo-*9H*-purin-6-amine (**6a**), expansion of the benzylic and aromatic region.

3-Benzyl-8-bromo-*3H*-purin-6-amine (30a)



30a

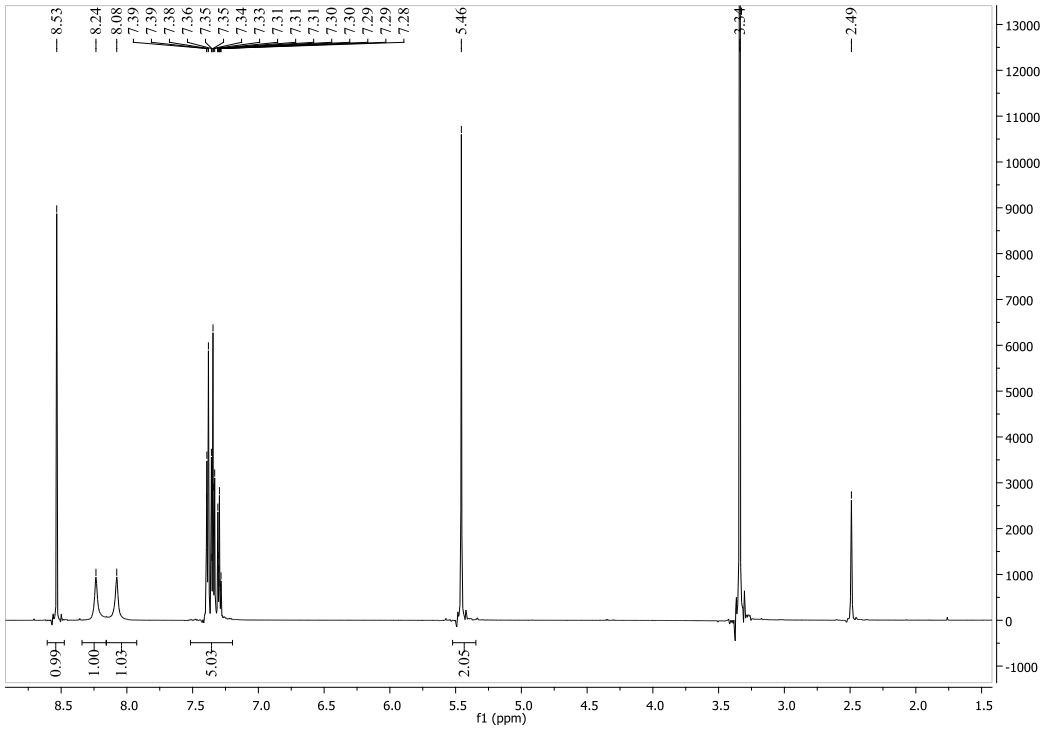
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.53 (s, 1H, H-2), 8.24 and 8.08 (br d, 2H, NH_2), 7.39 – 7.28 (m, 5H, H-12, H-13 and H-14), 5.46 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 153.6 (C-6), 149.8 (C-4), 144.0 (C-2), 139.3 (C-8), 135.8 (C-11), 128.7 (C-12 or C-13), 128.1 (C-14), 127.8 (C-12 or C-13), 121.5 (C-5), 52.0 (C-10).

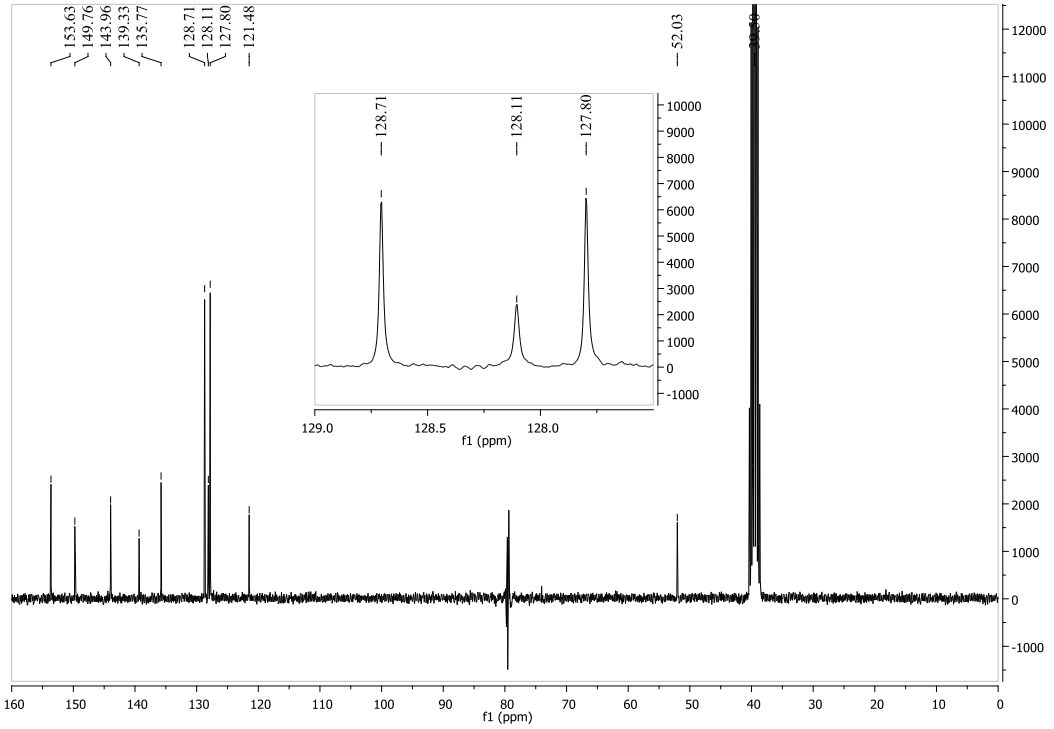
MS EI *m/z* (rel. %) 305/303 (20/20, M^+), 304/302 (18/14), 224 (30), 91 (100), 65 (11).

HR-MS Found 303.0125, calculated for $C_{12}H_{10}BrN_5$ 303.0120.

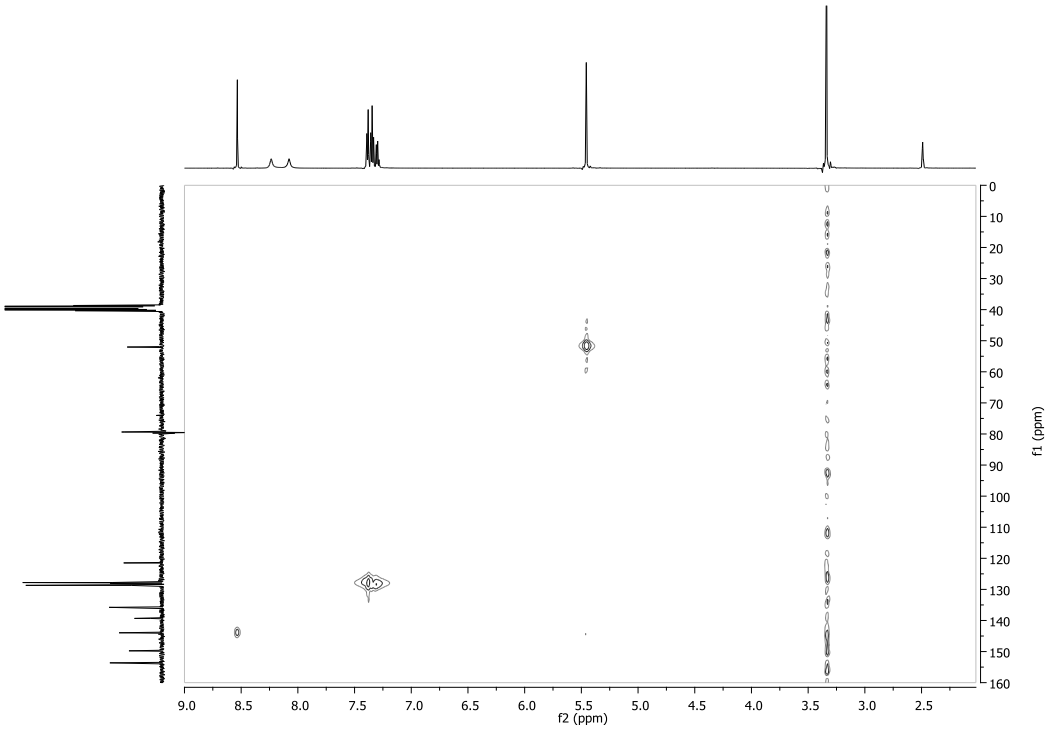
M.p. 233-235 °C.



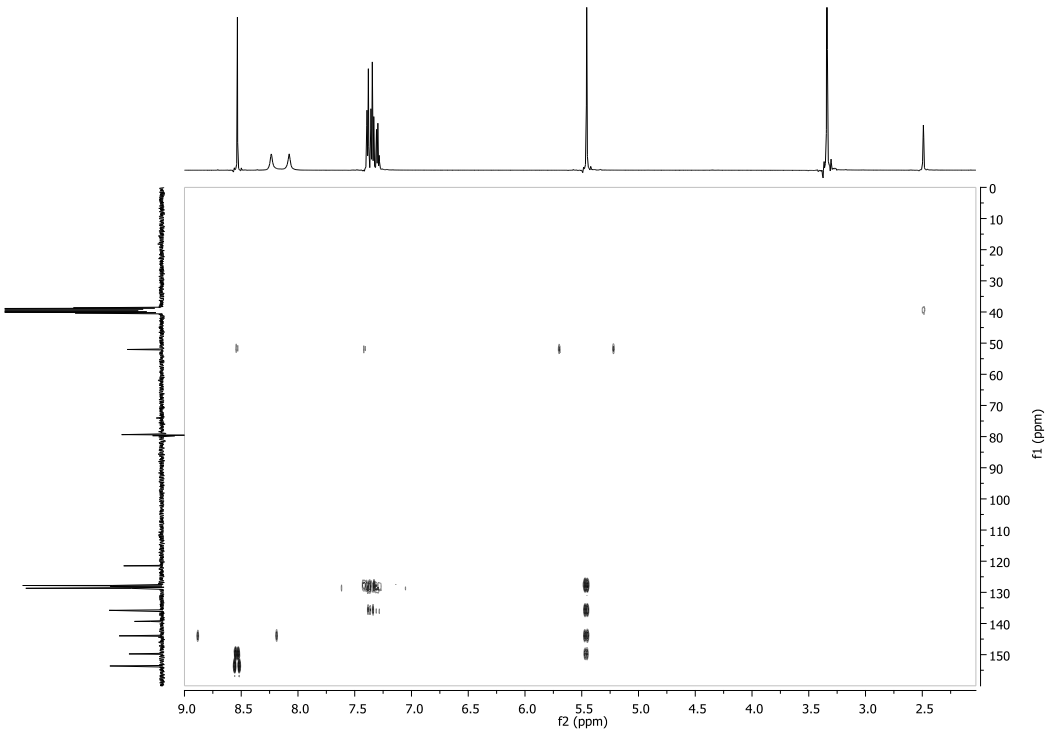
Spectrum 72. ^{1}H NMR of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**).



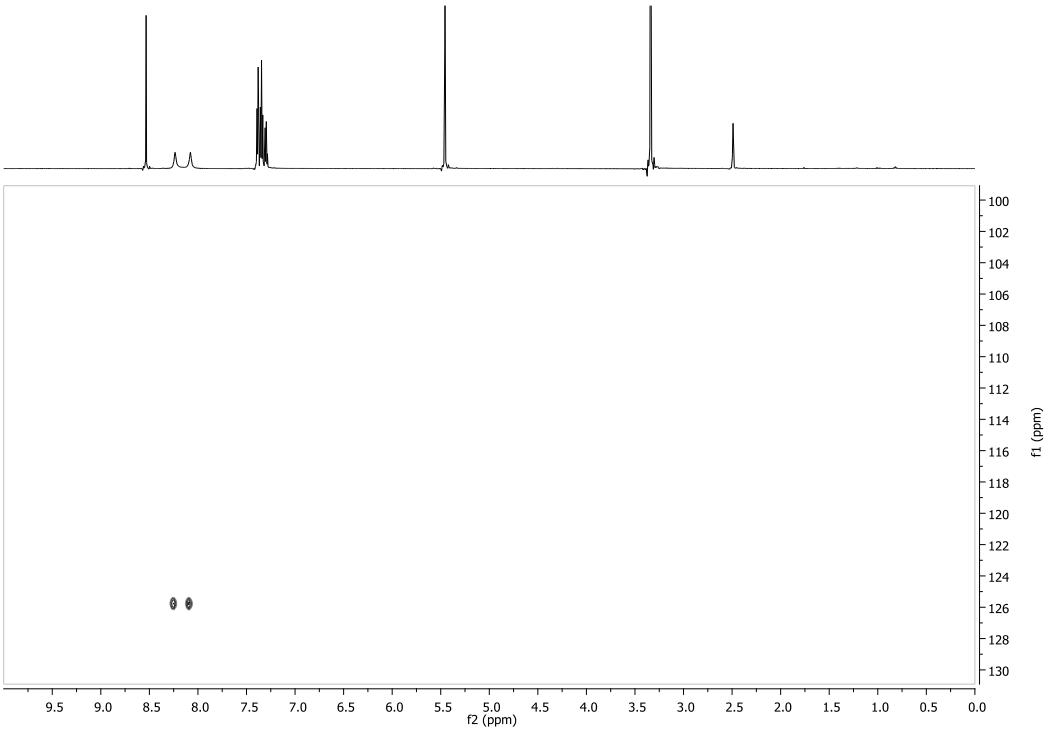
Spectrum 73. ^{13}C NMR of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**) with expansion of the phenyl region (inset).



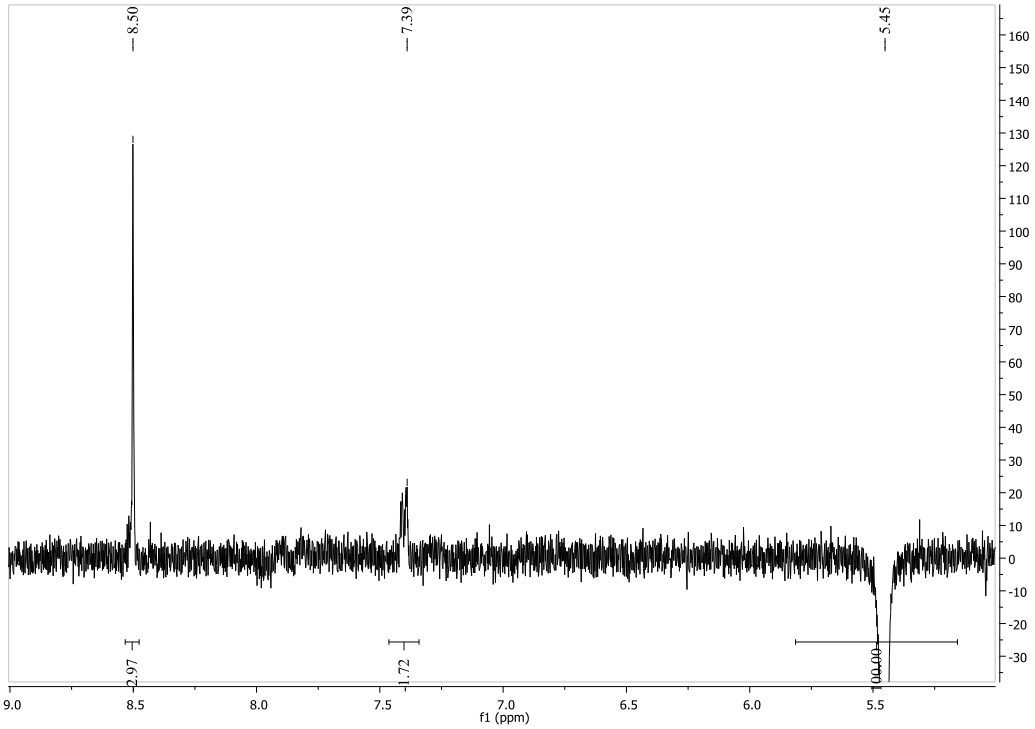
Spectrum 74. HMQC of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**).



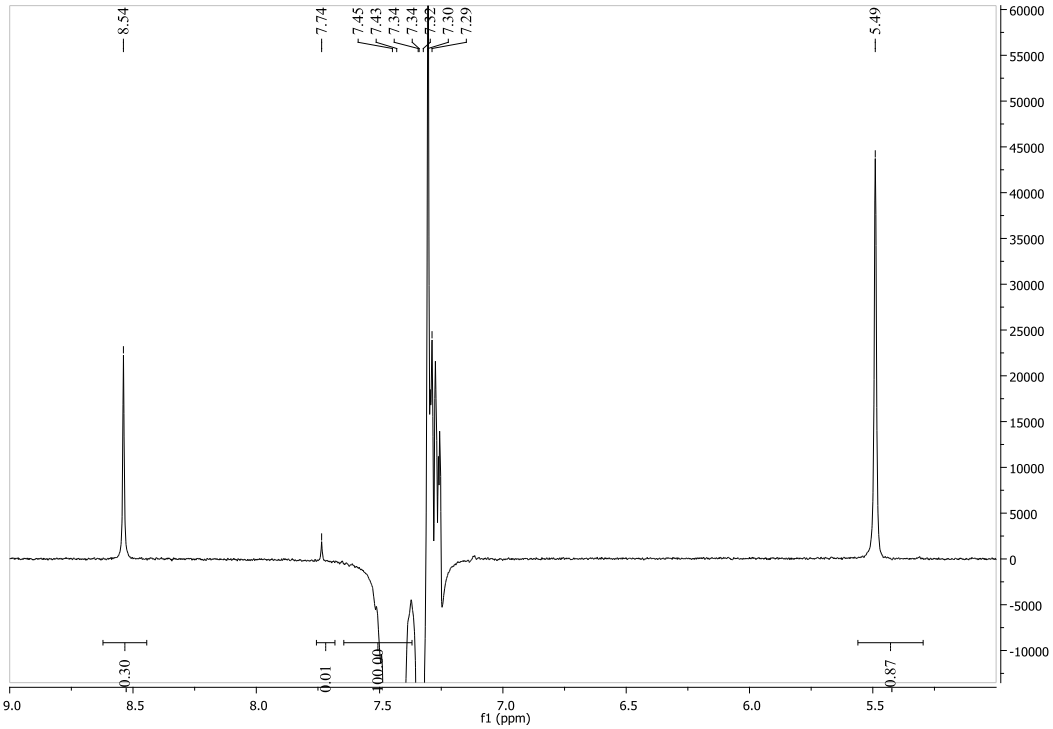
Spectrum 75. HMBC of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**).



Spectrum 76. ^{1}H-^{13}N HSQC of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**).

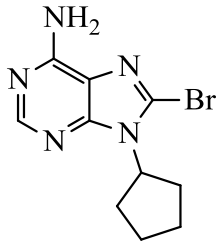


Spectrum 77. 1D selective NOE of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**), with irradiation of the protons in the methylene group.



Spectrum 78. 1D selective NOE of 3-Benzyl-8-bromo-*3H*-purin-6-amine (**30a**), with irradiation of the phenyl protons closest to the methylene group.

9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (6b)

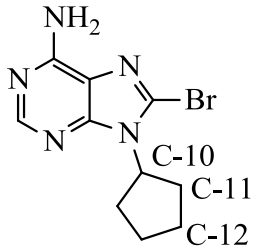


6b

Method 1: A mixture of bromine (0.20 mL, 3.9 mmol) and distilled water (15 mL) was poured over 9-cyclopentyladenine (**7b**) (108 mg, 0.531 mmol) and stirred at ambient temperature for 17 h with a condenser. The bromine and water mixture was evaporated first in the fumehood then *in vacuo.* The residue was purified by flash chromatography to give **6b** as a pale reddish powder (hexanes – 1:1 hexanes:ethyl acetate – ethyl acetate) (95 mg, 67%).

Method 2: LDA (2.52 mmol) in 5 mL dry THF was added dropwise to 9-cyclopentyladenine (**7b**) (103 mg, 0.507 mmol) dissolved in 10 mL dry THF at -78 °C and stirred for 1 hour under nitrogen atmosphere. 1,2-Dibromo-1,1,2,2-tetrachloroethane (500 mg, 3.07 mmol) in 1 mL dry THF was added dropwise and the reaction was stirred for a further 4 h. The reaction was quenched with 0.50 mL saturated ammonium chloride solution and the solvents evaporated *in vacuo*. The residue was purified using flash chromatography (1-5% methanol in dichloromethane) to give 102 mg of a 10:3 mixture of the expected product (**6b**) and the 8-chloro analogue (**8b**) (calculated yields from NMR = 58% and 17%, respectively).

9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (6b)



6b

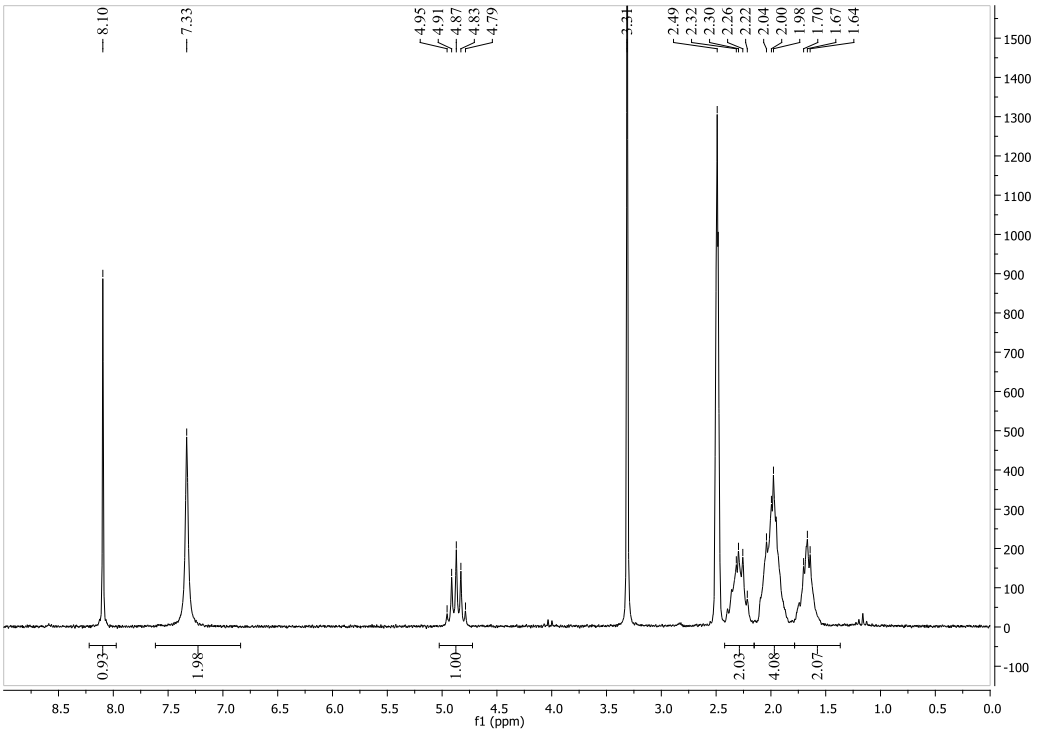
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.10 (s, 1H, H-2), 7.32 (s, 2H, NH_2), 4.873 (quintet, J = 8.4 Hz, H-10), 2.37 – 2.27 (m, 2H, H-11 and H-12), 2.25 – 1.91 (m, 4H, H-11 and H-12), 1.90 – 1.67 (m, 2H, H-11 and H-12).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.8 (C-6), 152.2 (C-2), 150.4 (C-4), 126.6 (C-8), 119.6 (C-5), 57.8 (C-10), 30.0 (C-11 or C-12), 24.4 (C-11 or C-12).

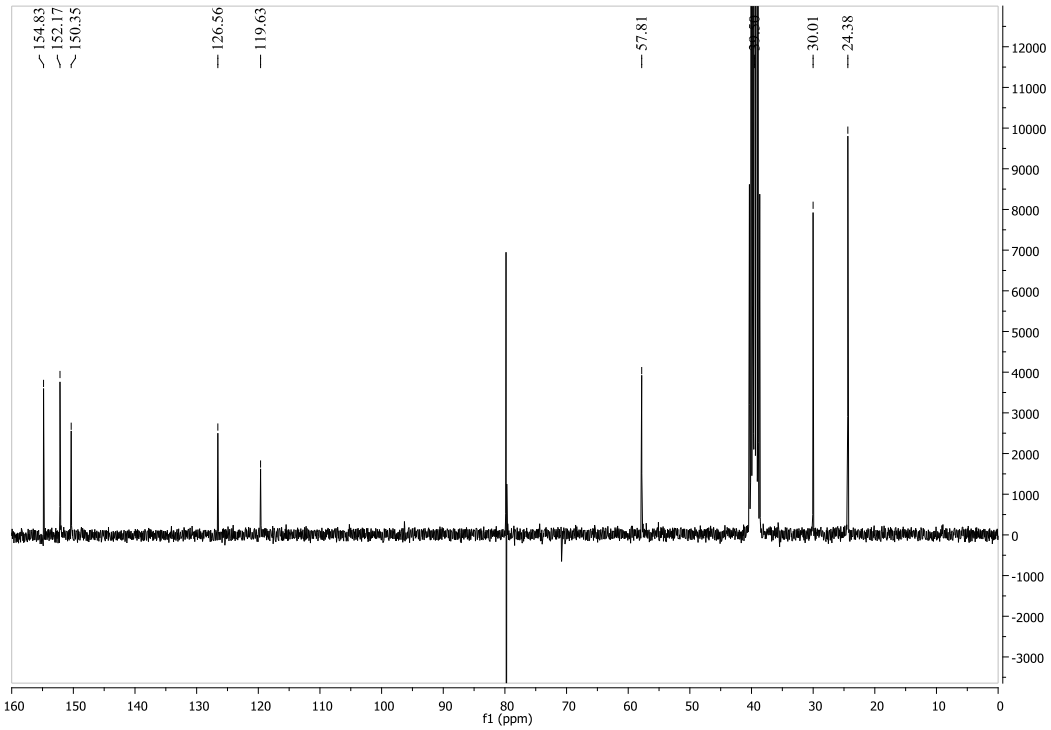
MS EI *m/z* (rel. %) 283/281 (17/17, M^+), 242/40 (13/13), 215/213 (99/100), 202 (15), 188/186 (16/16).

HR-MS Found 281.0273, calculated for $C_{10}H_{12}BrN_5$ 281.0276.

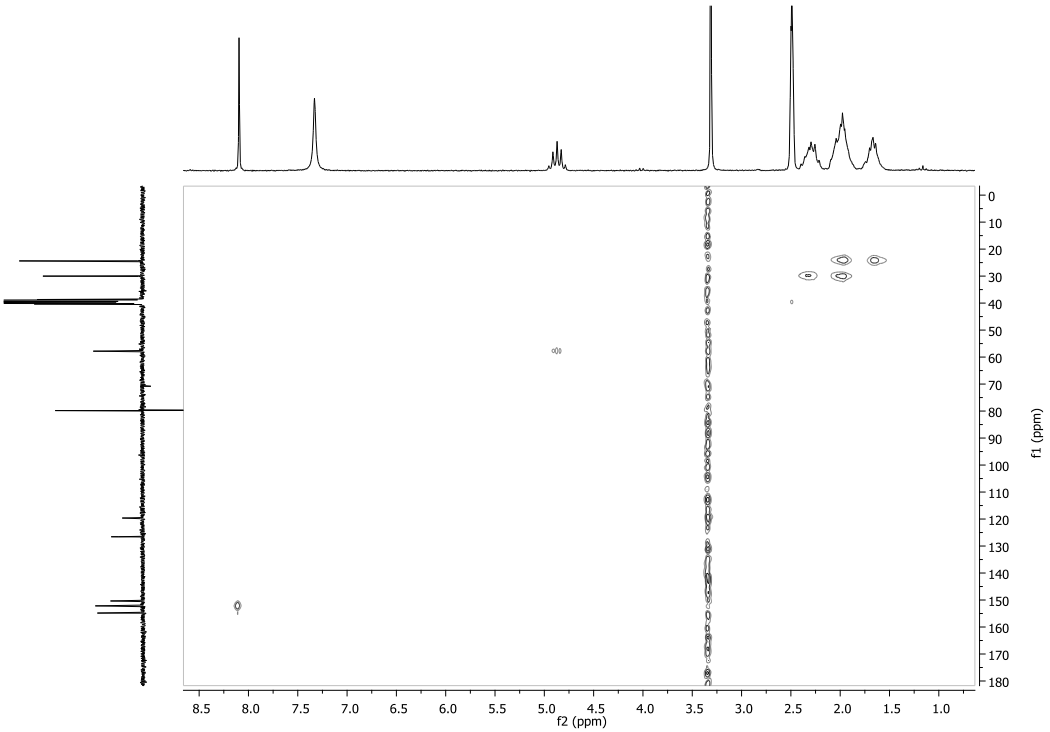
M.p. 186-189 °C (lit.[69] = 193 °C).



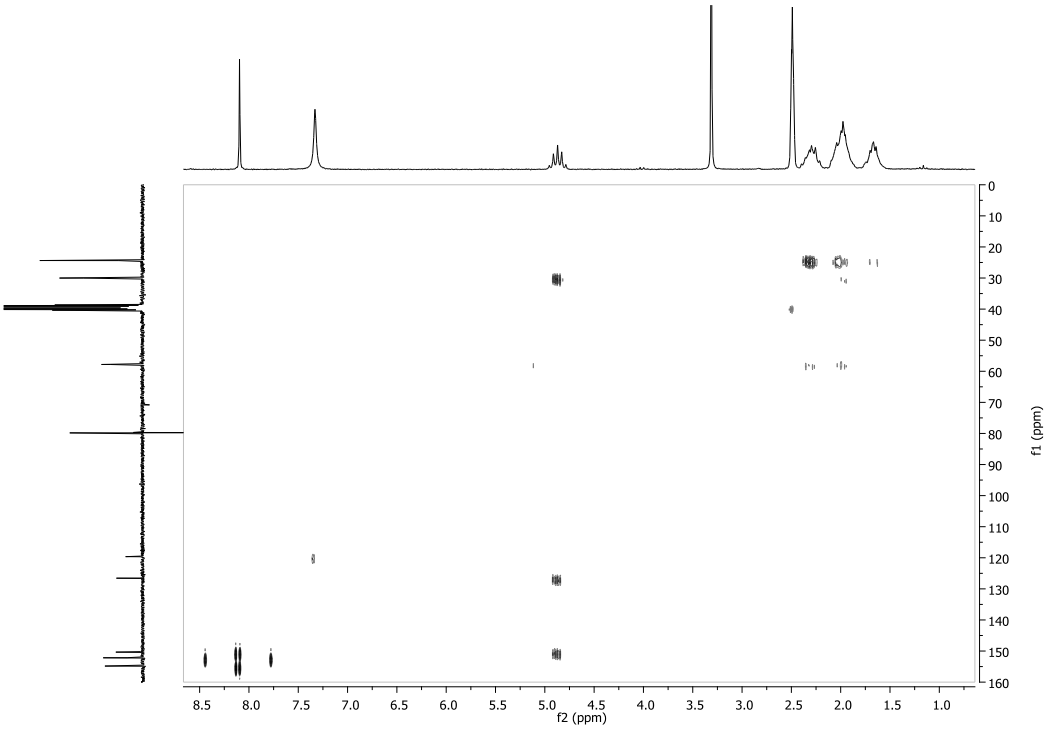
Spectrum 79. ^{1}H NMR of 9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (**6b**).



Spectrum 80. ^{13}C NMR of 9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (**6b**).

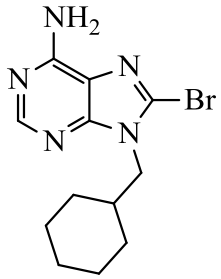


Spectrum 81. HMQC of 9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (**6b**).



Spectrum 82. HMQC of 9-Cyclopentyl-8-bromo-*9H*-purin-6-amine (**6b**).

9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (6c)



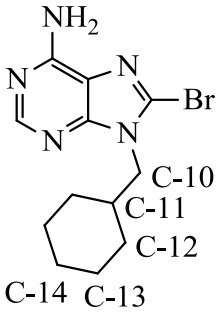
6c

Method 1: A mixture of 8-bromoadenine (**5**) (217 mg, 1.06 mmol), DMF (5 mL) and potassium carbonate (280 mg, 2.01 mmol) was stirred at ambient temperature under a nitrogen atmosphere. (Bromomethyl)cyclohexane (0.28 mL, 2.0 mmol) was added and the mixture stirred for 20 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography to give **6c** as a colourless powder (0-10% methanol in dichloromethane) (43 mg, 13%).

Method 2: A mixture of bromine (0.20 mL, 3.9 mmol) and distilled water (15 mL) was poured over 9-(cyclohexylmethyl)adenine (**7c**) (146 mg, 0.631 mmol) and stirred at ambient temperature for 17 h with a condenser. The bromine and water mixture was evaporated first in the fumehood then *in vacuo.* The residue was purified by flash chromatography to give **6c** as a pale reddish powder (0:1 – 1:0 hexanes:ethyl acetate) (126 mg, 66%).

Method 3: LDA (2.52 mmol) in 5 mL dry THF was added dropwise to 9-(cyclohexylmethyl)adenine (**7c**) (118 mg, 0.510 mmol) dissolved in 10 mL dry THF at -78 °C and stirred for 1 hour under nitrogen atmosphere. 1,2-dibromotetrachloroethane (495 mg, 3.04 mmol) in 1 mL dry THF was added dropwise and the reaction was stirred for a further 4 h. The reaction was quenched with 0.50 mL saturated ammonium chloride solution and the solvents evaporated *in vacuo*. The residue was purified using flash chromatography (1-5% methanol in dichloromethane) to give 100 mg of a 5:2 mixture of the expected product (**6c**) and the 8-chloro analogue (**8c**) (calculated yields from NMR = 57% and 23%, respectively).

9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (6c)



6c

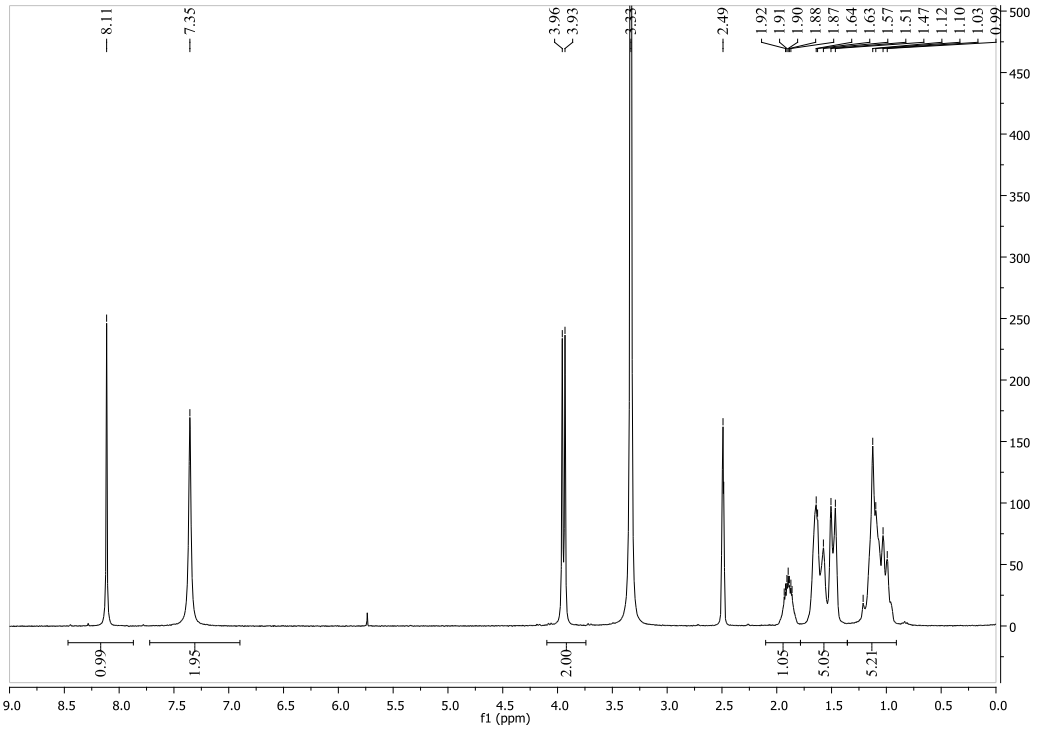
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.11 (s, 1H, H-2), 7.35 (br s, 2H, NH_2), 3.94 (d, 2H, C-10, J = 7.5 Hz), 1.90 (ddd, J = 10.6, 7.3, 3.4 Hz, 1H, H-11), 1.78 – 1.36 (m, 5H, H-12, H-13 and H-14), 1.36 – 0.91 (m, 5H, H-12, H-13 and H-14).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.7 (C-6), 152.8 (C-2), 151.0 (C-4), 126.6 (C-8), 118.9 (C-5), 49.5 (C-10), 37.3 (C-11), 30.0 (C-12), 25.7 (C-14), 25.1 (C-13).

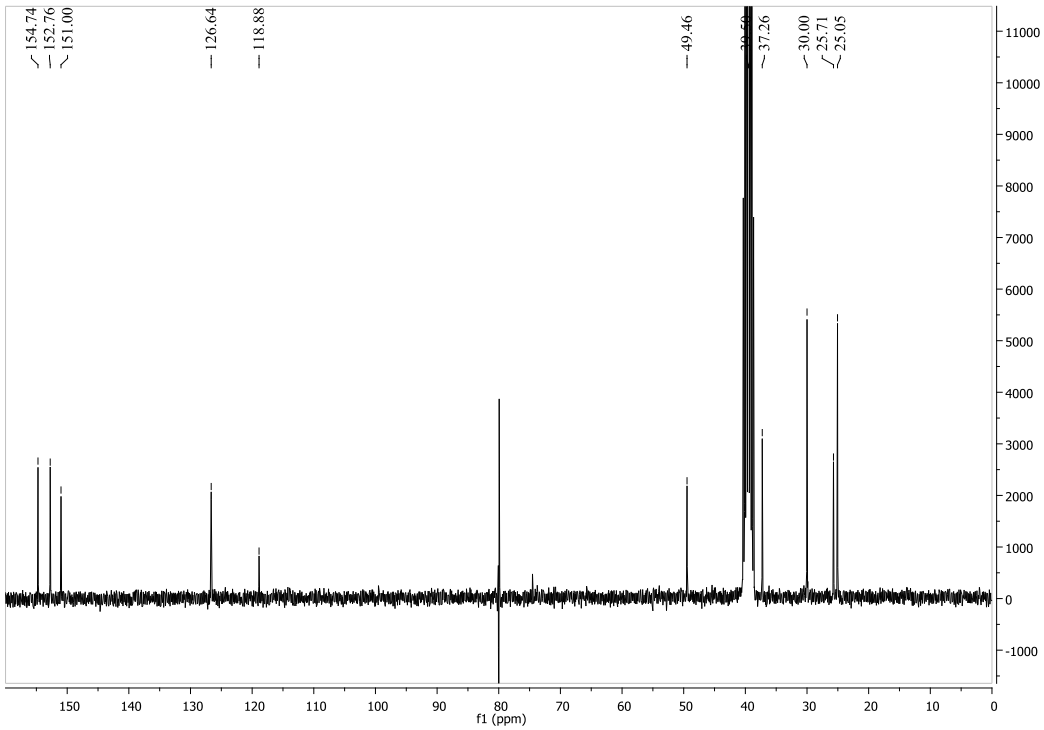
MS EI *m/z* (rel. %) 311/309 (2/2, M^+), 231 (39), 230 (100), 227 (10), 215/213 (28/26), 148 (40).

HR-MS Found 309.0587, calculated for $C_{12}H_{16}BrN_5$ 309.0589.

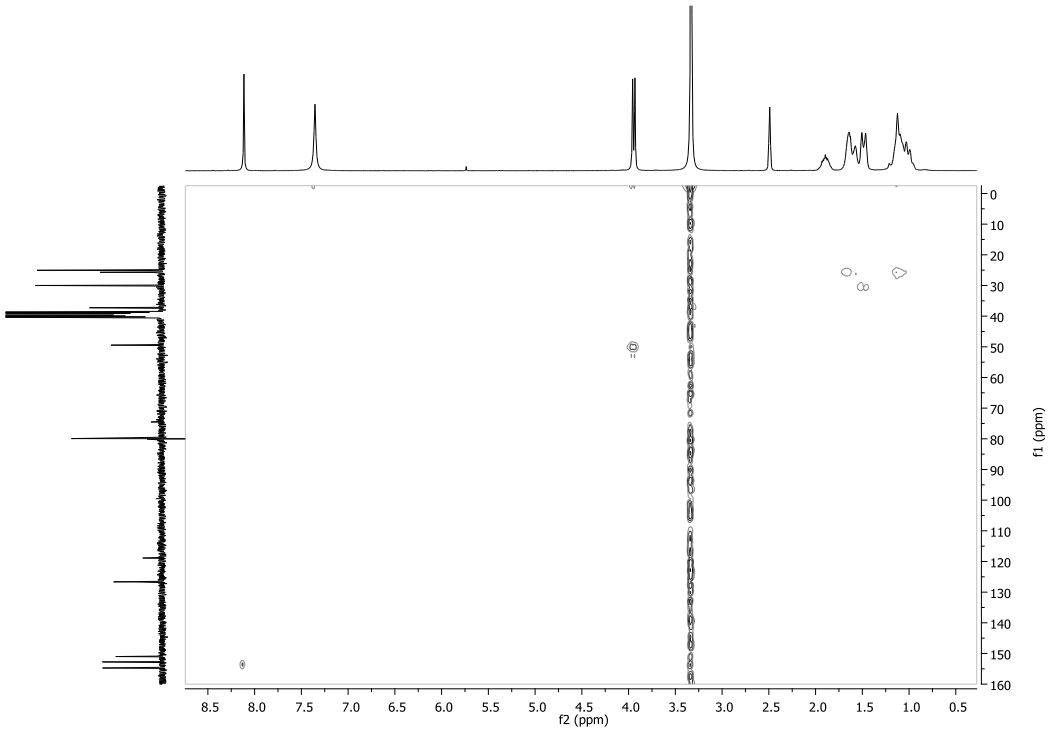
M.p. 228-230 °C.



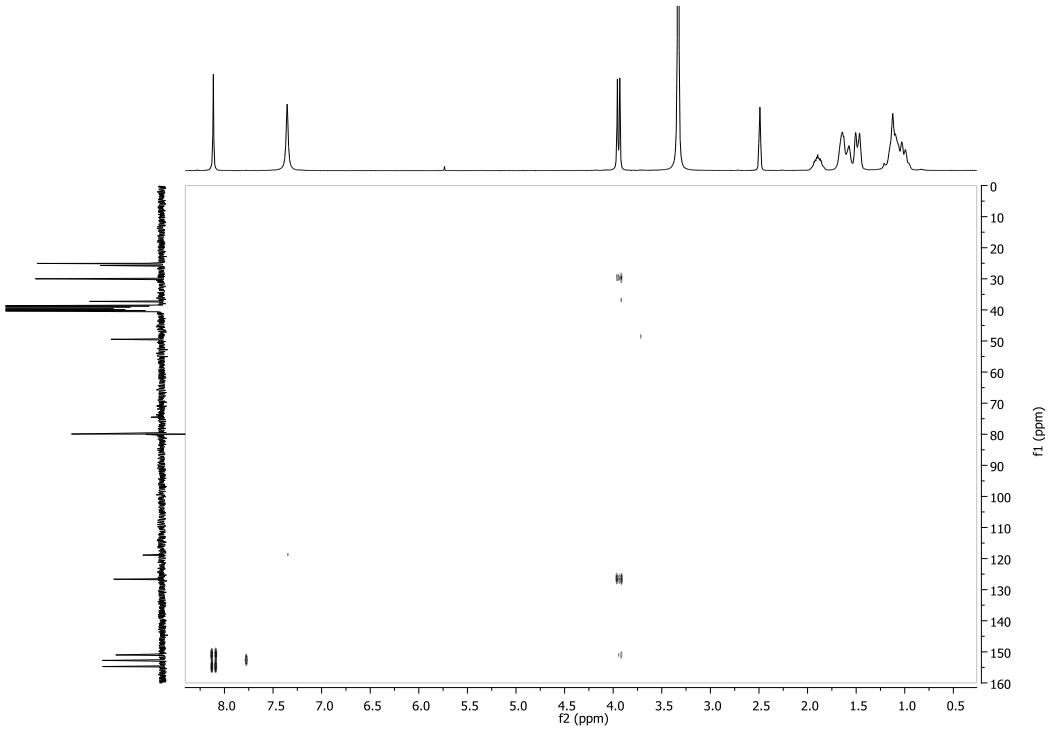
Spectrum 83. ^{1}H NMR of 9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (**6c**).



Spectrum 84. ^{13}C NMR of 9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (**6c**).



Spectrum 85. HMQC of 9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (**6c**).



Spectrum 86. HMBC of 9-(Cyclohexylmethyl)-8-bromo-*9H*-purin-6-amine (**6c**).

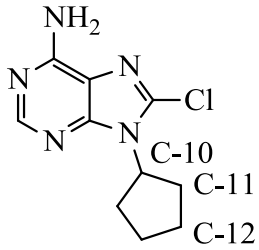
General procedure for lithiation and subsequent halogenation of 9-substituted adenines

Lithium diisopropylamide (LDA) was generated from *n*-BuLi in hexanes and dry diisopropylamine by cooling approximately 2.6 mmol diisopropylamine in dry THF to -78 °C under an inert atmosphere. *n*-BuLi in hexanes (approximately 2.5 mmol) was added dropwise and the mixture was stirred for one hour under these conditions.

The appropriate 9-substituted adenine (0.5 mmol) was dissolved in dry THF (10 mL for **7b** and **7c** and 20 mL for **7a**) and added dropwise to the LDA in 5 mL dry THF at -78 °C under an argon atmosphere. This reaction mixture was stirred for one hour at this temperature. The halogenating agent (dibromotetrachloroethane or hexachloroethane) (1.5 mmol) in 2 mL dry THF was added dropwise and the reaction was stirred for a further 4 h. The reaction was quenched with 0.50 mL saturated ammonium chloride solution and allowed to come to ambient temperature.

The mixture was transferred to a separatory funnel and a further 15 mL ammonium chloride solution was added. The organic phase was removed and the water phase was extracted with 3 x 15 mL ethyl acetate. The organic phases were combined and dried with 15 mL saturated sodium chloride solution and the solvent evaporated *in vacuo*. The residue was purified using flash chromatography (0:1 – 1:0 ethyl acetate:dichloromethane) to give compounds **8b – 8d** as colourless powders.

8-Chloro-9-cyclopentyl-*9H*-purin-6-amine (8b)



8b

Yield 82 mg, 69%

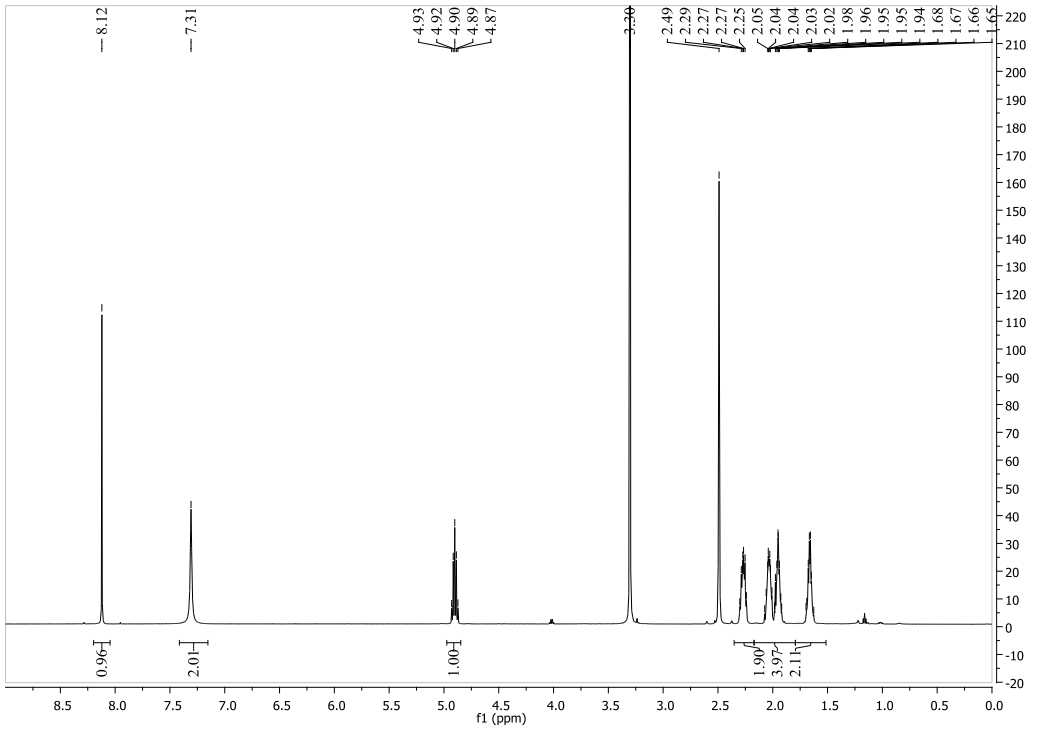
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.12 (s, 1H, H-2), 7.31 (s, 2H, NH_2), 4.90 (p, J = 8.6 Hz, 1H, H-10), 2.35 – 2.17 (m, 2H, H-11 and H-12), 2.17 – 1.79 (m, 4H, H-11 and H-12), 1.79 – 1.51 (m, 2H, H-11 and H-12).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.9 (C-6), 152.4 (C-2), 150.1 (C-4), 136.4 (C-8), 117.9 (C-5), 56.6 (C-10), 30.0 (C-11), 24.4 (C-12).

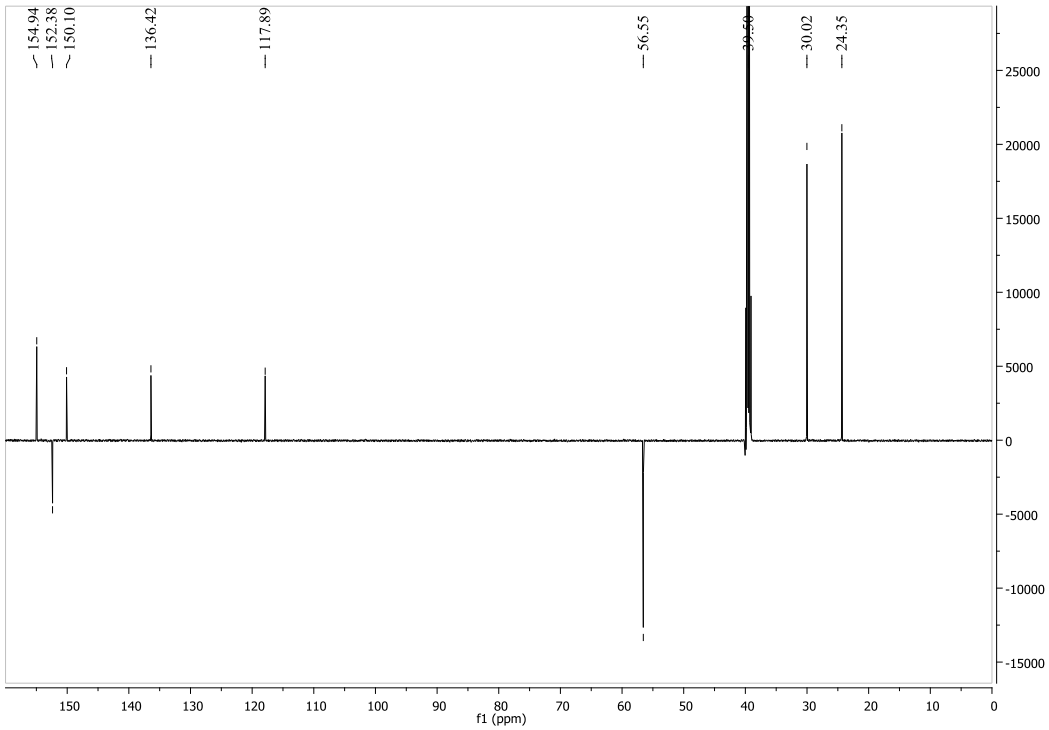
MS EI *m/z* (rel. %) 239/237 (5/15, M^+), 202 (7), 198/196 (5/15), 171/169 (34/100), 144/142 (7/22).

HR-MS Found 237.0782, calculated for $C_{10}H_{12}ClN_5$ 237.0781.

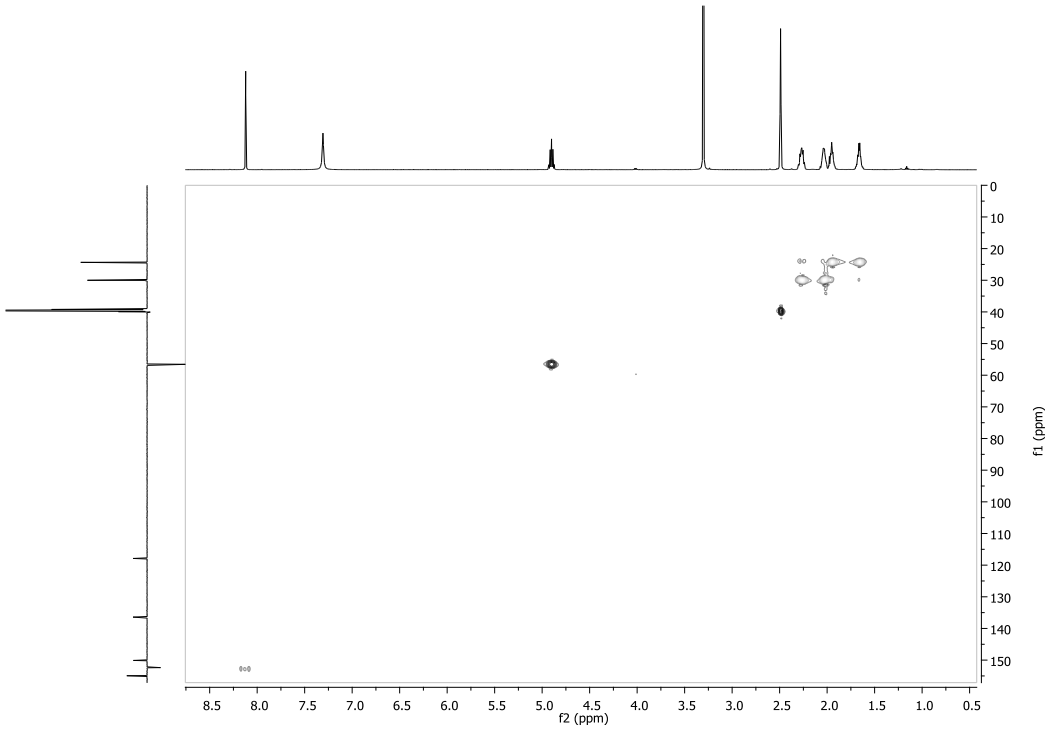
M.p. 181-183 °C.



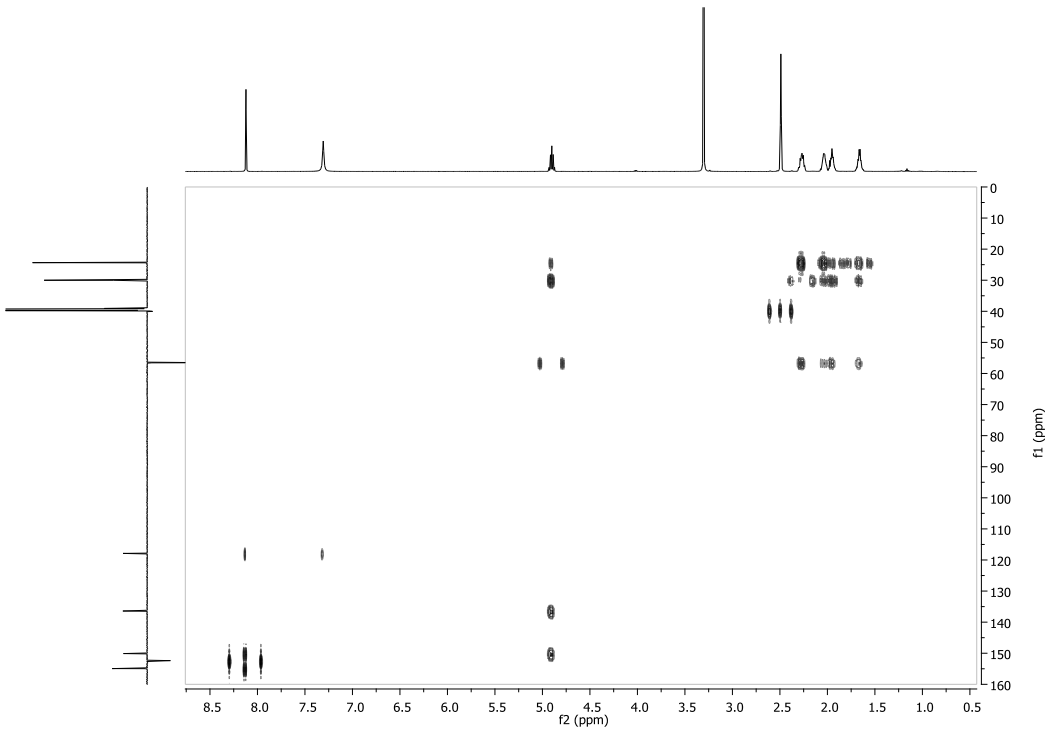
Spectrum 87. ^{1}H NMR of 8-Chloro-9-cyclopentyl-*9H*-purin-6-amine (**8b**).



Spectrum 88. ^{13}C APT NMR of 8-Chloro-9-cyclopentyl-*9H*-purin-6-amine (**8b**).

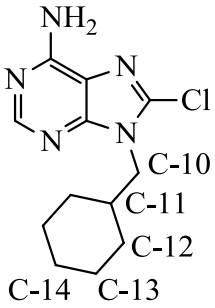


Spectrum 89. HSQC of 8-Chloro-9-cyclopentyl-*9H*-purin-6-amine (**8b**).



Spectrum 90. HMBC of 8-Chloro-9-cyclopentyl-*9H*-purin-6-amine (**8b**).

8-Chloro-9-(cyclohexylmethyl)-*9H*-purin-6-amine (8c)



8c

Yield 90 mg, 68%

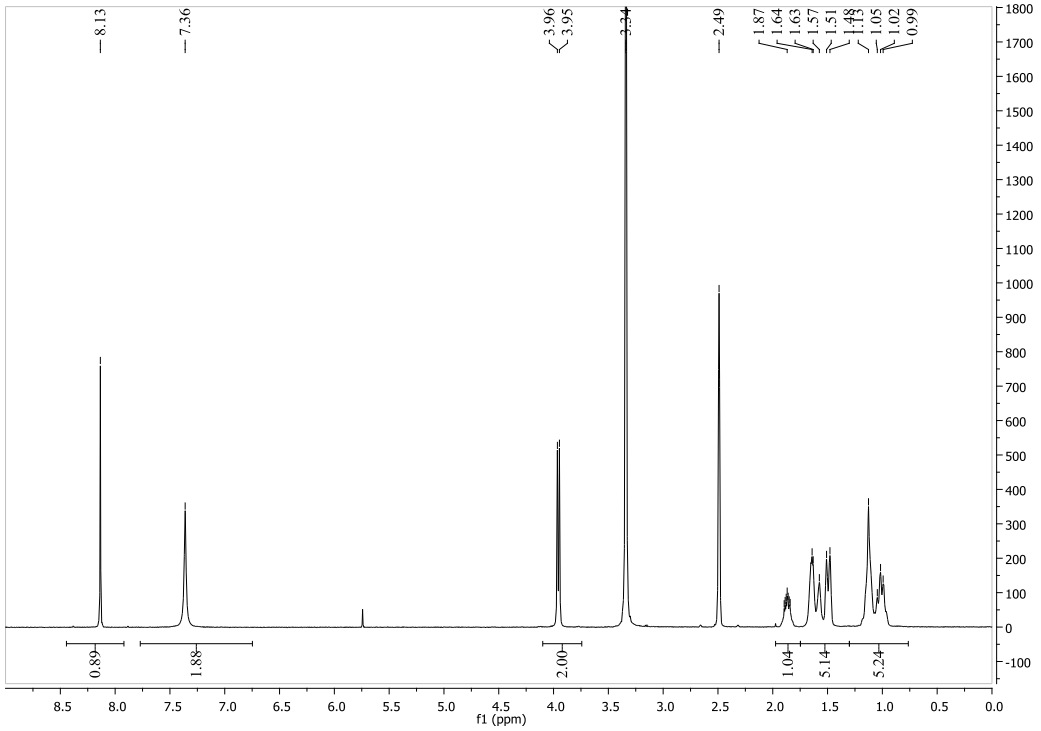
^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.13 (s, 1H, H-2), 7.36 (s, 2H, NH_2), 3.96 (d, J = 7.4 Hz, 2H, H-10), 1.87 (ddd, J = 10.9, 7.4, 3.5 Hz, 1H, H-11), 1.75 – 1.30 (m, 5H, H-12, H-13 and H-14), 1.13 – 0.99 (m, 5H, H-12, H-13 and H-14).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.8 (C-6), 152.9 (C-2), 150.6 (C-4), 136.8 (C-8), 117.4 (C-5), 48.7 (C-10), 37.1 (C-11), 29.9 (C-12 or C-13), 25.7 (C-14), 25.0 (C-12 or C-13).

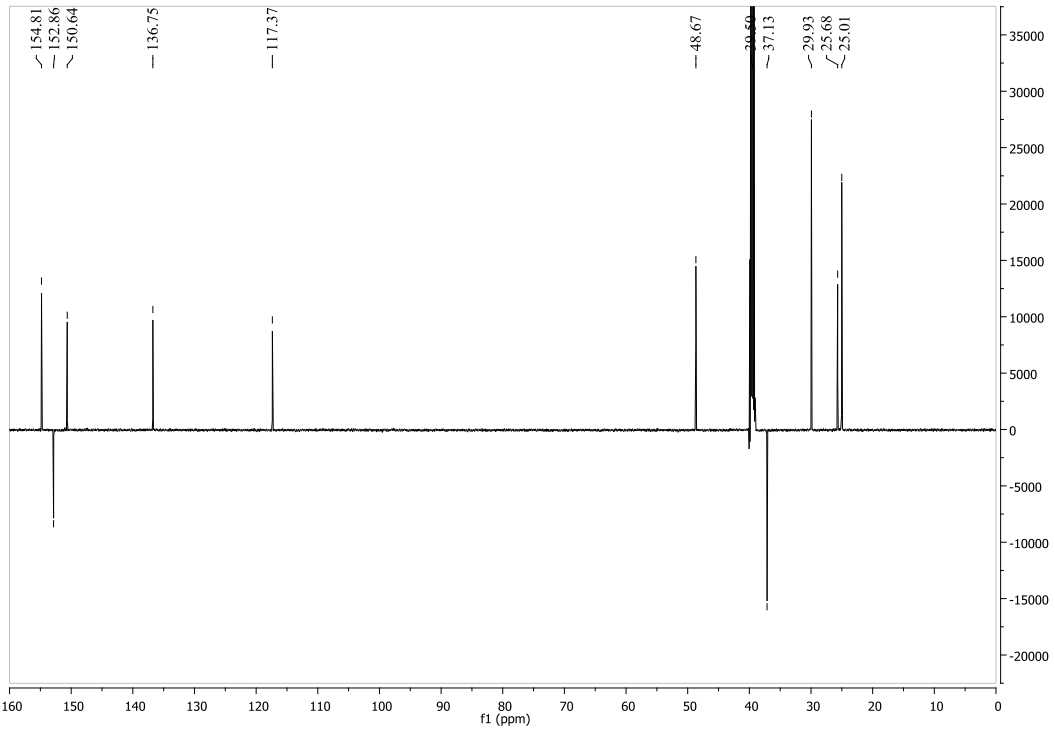
MS EI *m/z* (rel. %) 267/265 (2/8, M^+), 230 (100), 185/183 (7/21), 184/182 (8/19), 171/169 (14/43), 148 (15), 142 (8).

HR-MS Found 265.1098, calculated for $C_{12}H_{16}ClN_5$ 265.1094.

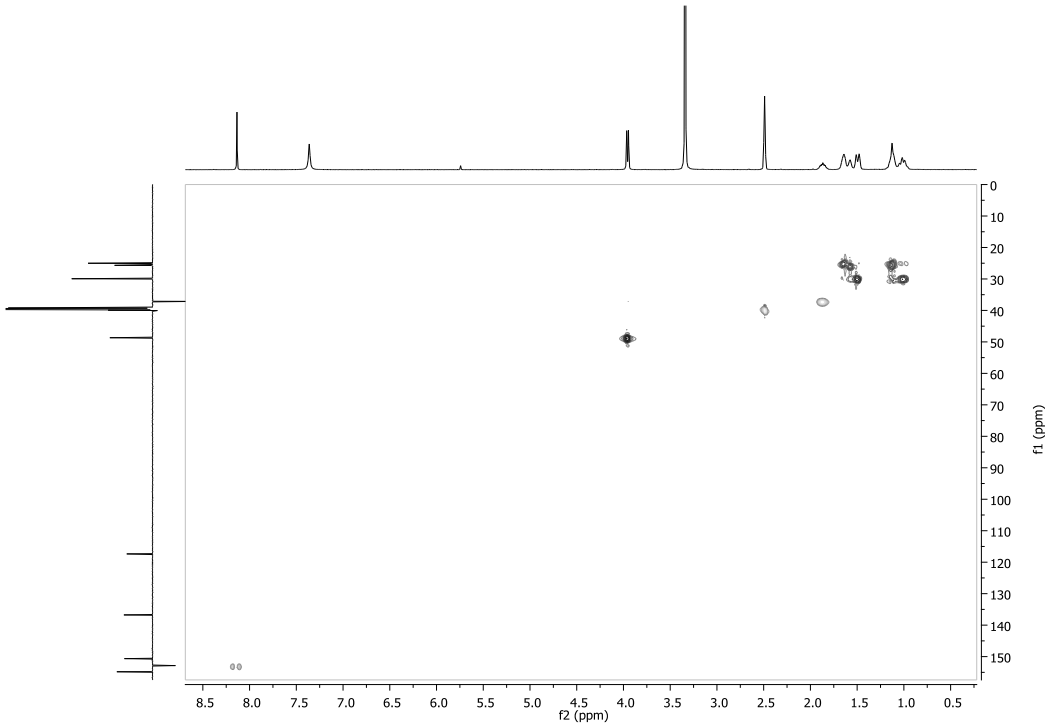
M.p. 229-230 °C.



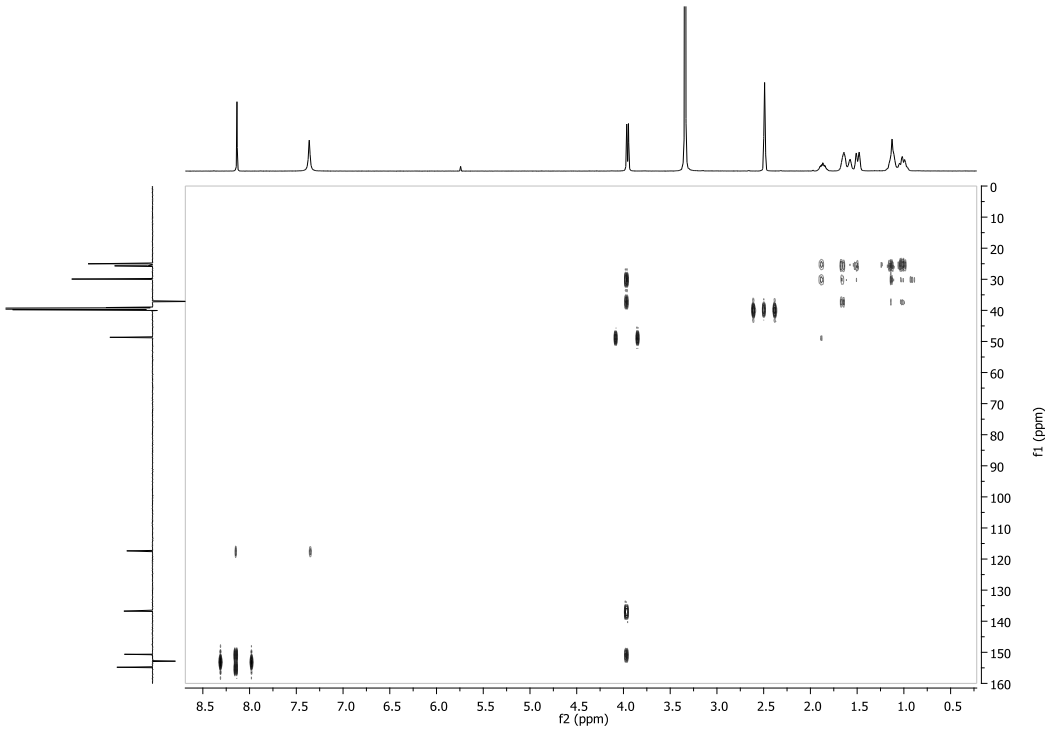
Spectrum 91. ^{1}H NMR of 8-Chloro-9-(cyclohexylmethyl)-*9H*-purin-6-amine (**8c**).



Spectrum 92. ^{13}C APT NMR of 8-Chloro-9-(cyclohexylmethyl)-*9H*-purin-6-amine (**8c**).

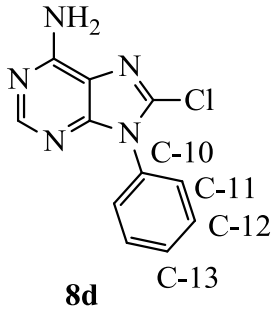


Spectrum 93. HSQC of 8-Chloro-9-(cyclohexylmethyl)-*9H*-purin-6-amine (**8c**).



Spectrum 94. HMBC of 8-Chloro-9-(cyclohexylmethyl)-*9H*-purin-6-amine (**8c**).

8-Chloro-9-phenyl-*9H*-purin-6-amine (8d)



8d

Yield 89 mg, 67%

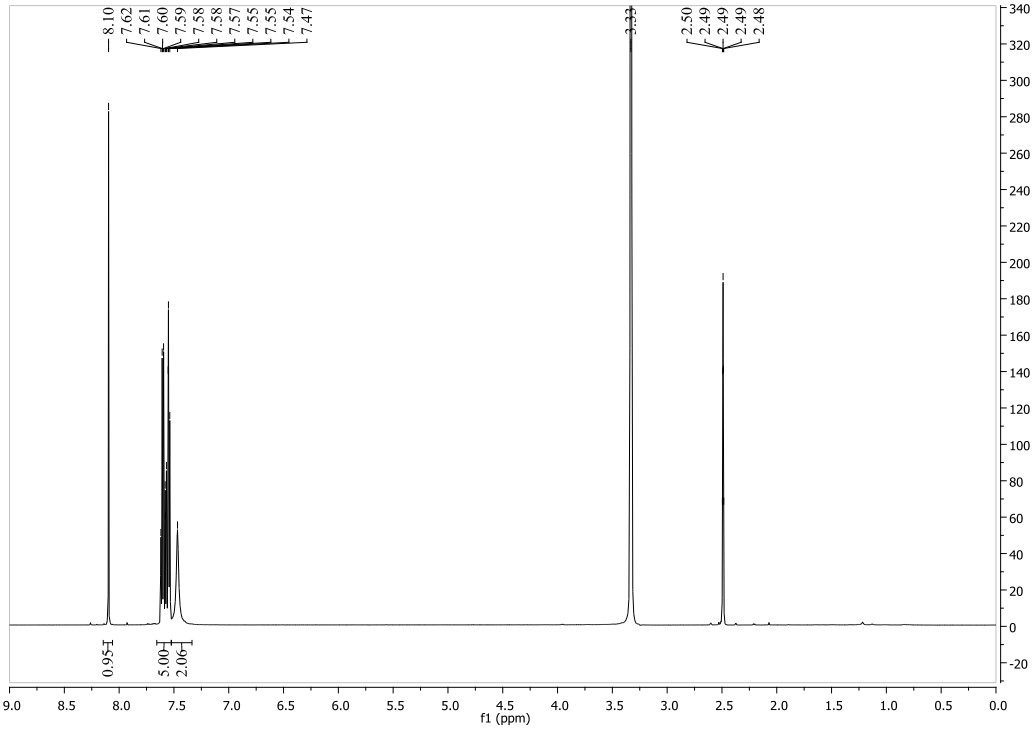
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.10 (s, 1H, H-2), 7.66 – 7.52 (m, 5H, H-10, H-11 and H-12), 7.47 (br s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 155.0 (C-4 or C-6), 153.4 (C-2), 151.3 (C-4 or C-6), 136.3 (C-8), 133.0 (C-10), 129.44 (C-13), 129.40 (C-11 or C-12), 127.8 (C-11 or C-12), 117.4 (C-5).

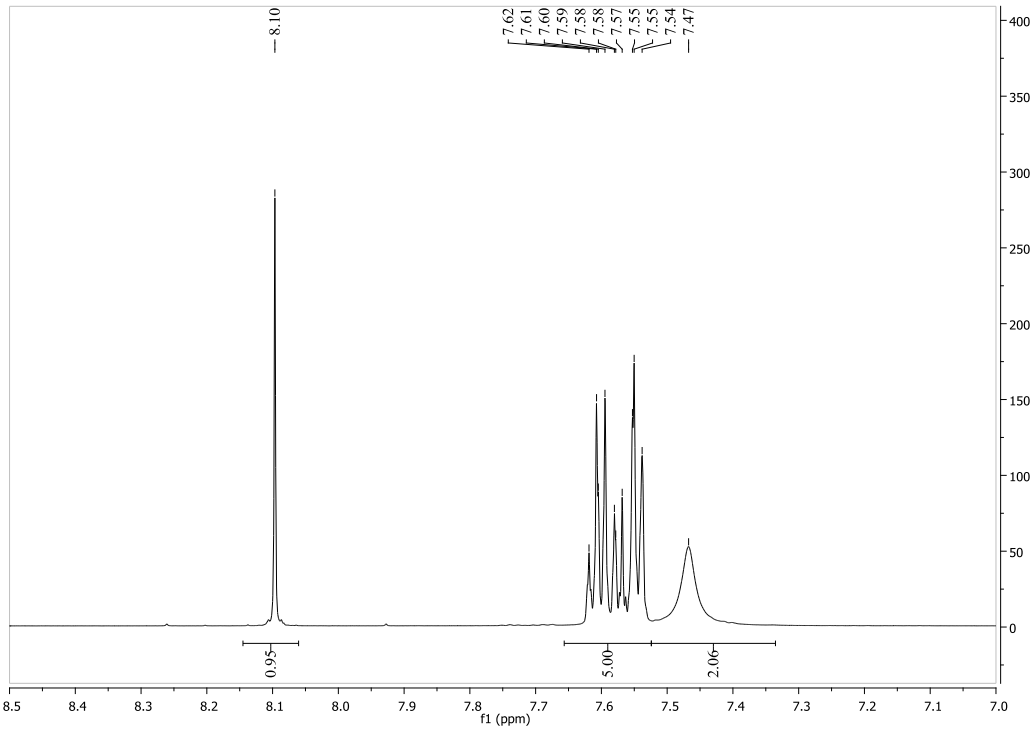
MS EI *m/z* (rel. %) 247/245 (36/100, M^+), 218 (13), 183 (10), 156 (6), 103 (6), 77 (16).

HR-MS Found 245.0469, calculated for $C_{11}H_8ClN_5$ 245.0468.

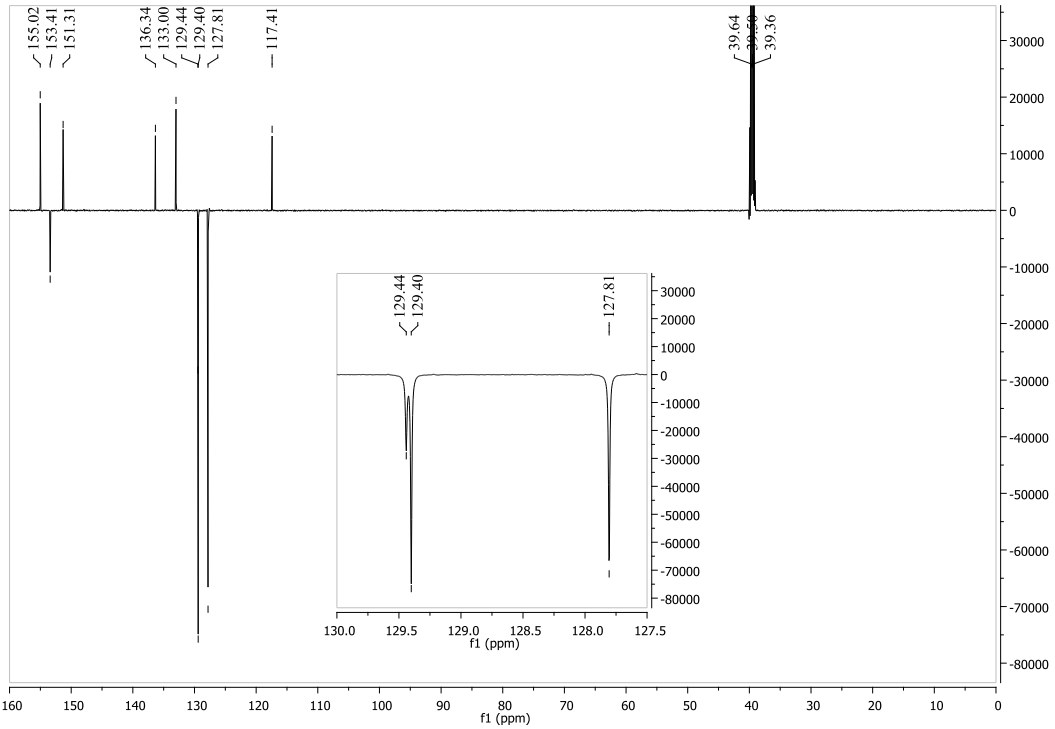
M.p. 225-226 °C.



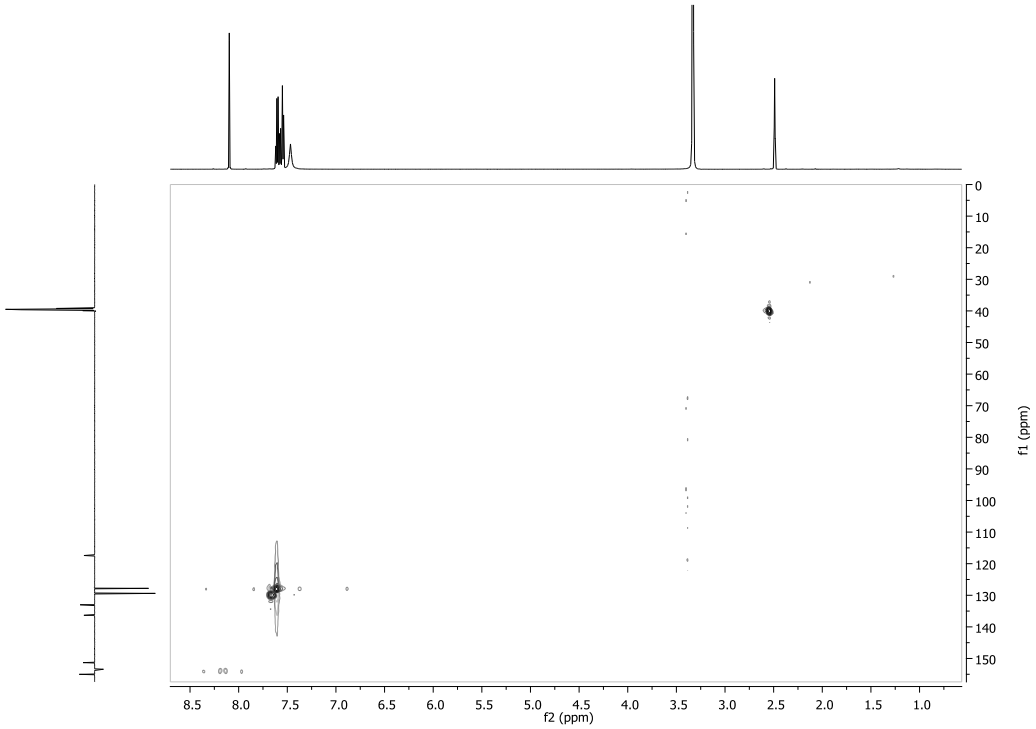
Spectrum 95. ^{1}H NMR of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**).



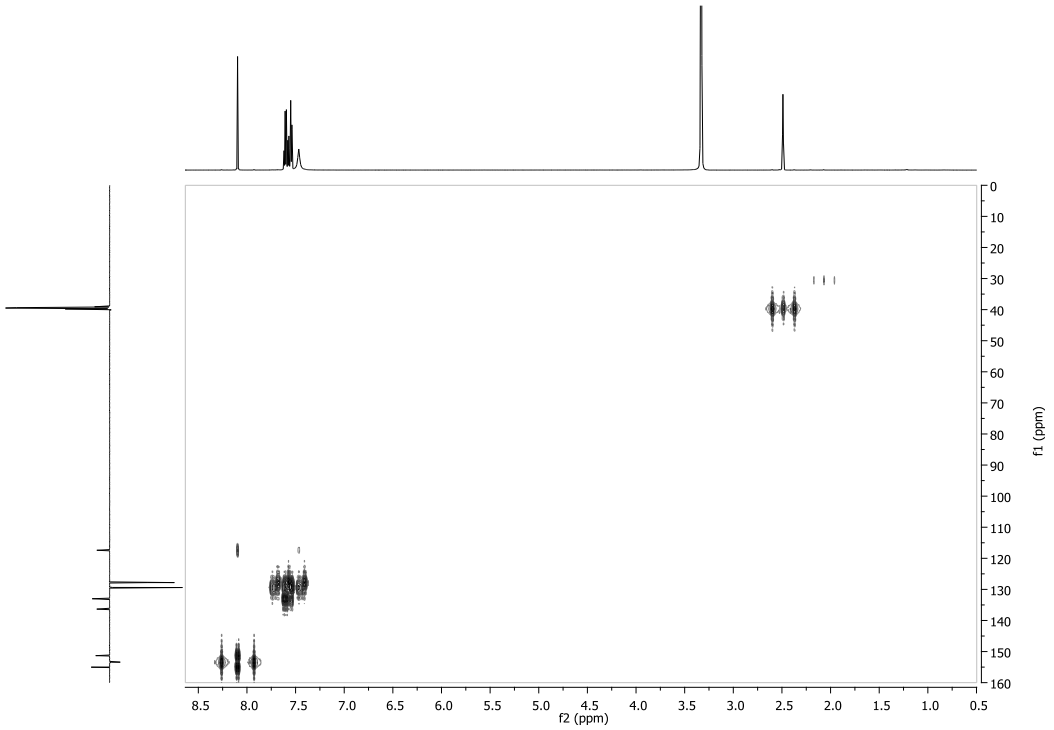
Spectrum 96. ^{1}H NMR of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**), expansion of the aromatic region.



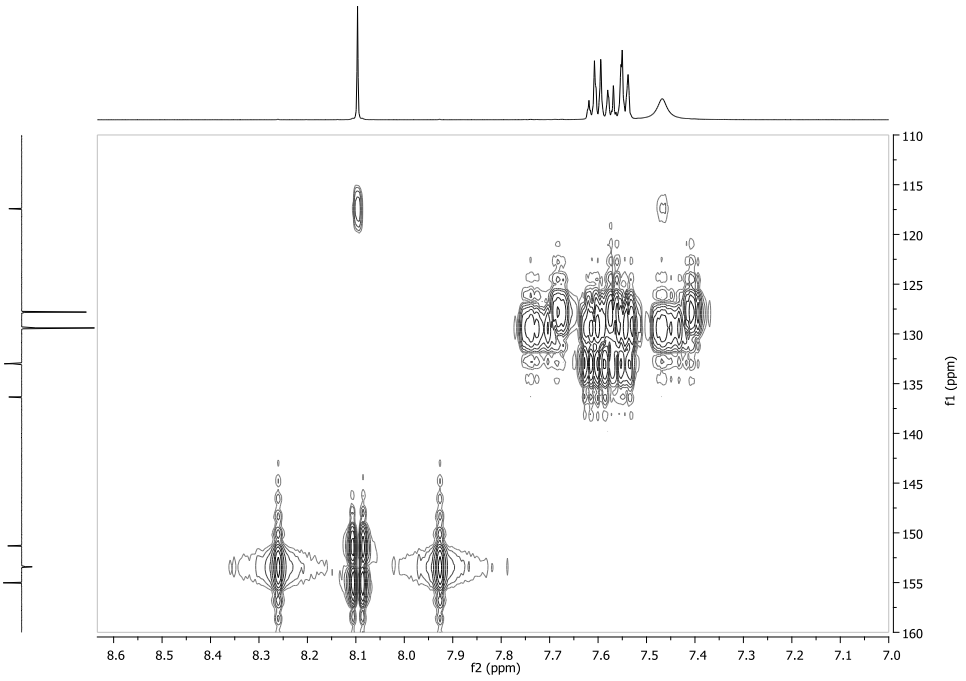
Spectrum 97. ^{13}C NMR of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**), with expansion of the phenyl region (inset).



Spectrum 98. HSQC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**).

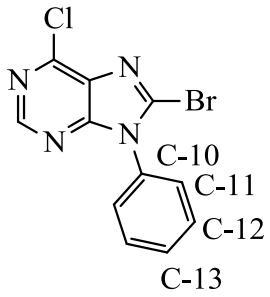


Spectrum 99. HMBC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**).



Spectrum 100. HMBC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**8d**), expansion of the aromatic region.

8-Bromo-6-chloro-9-phenyl-*9H*-purine (44) and By-product (45)



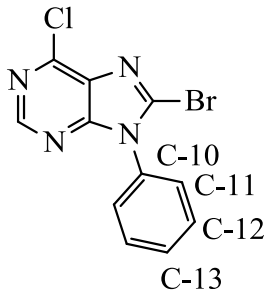
44

Lithium diisopropylamide (LDA) was generated from *n*-BuLi in hexanes and dry diisopropylamine by cooling diisopropylamine (0.77 mmol) in dry THF to -78 °C under an inert atmosphere. *n*-BuLi in hexanes (0.70 mmol) was added dropwise and the mixture was stirred for one hour under these conditions.

6-Chloro-9-phenylpurine (**14**) (110 mg, 0.481 mmol) was dissolved in 5 mL dry THF and added dropwise to the LDA in 5 mL dry THF at -78 °C under an argon atmosphere. This mixture was stirred for one hour under these conditions. This reaction mixture was stirred for one hour at this temperature. 1,2-Dibromo-tetrachloroethane (331 mg, 1.02 mmol) in 1 mL dry THF was added dropwise and the reaction was stirred for a further 2 h.

The reaction was quenched with 0.50 mL saturated ammonium chloride solution and allowed to come to ambient temperature. The solvent was evaporated *in vacuo* and the residue was purified using flash chromatography (1:3 ethyl acetate:hexanes) to give **44** as a colourless powder (90 mg, 61%) and 15 mg of impure by-product (**45**).

8-Bromo-6-chloro-9-phenyl-*9H*-purine (44)



44

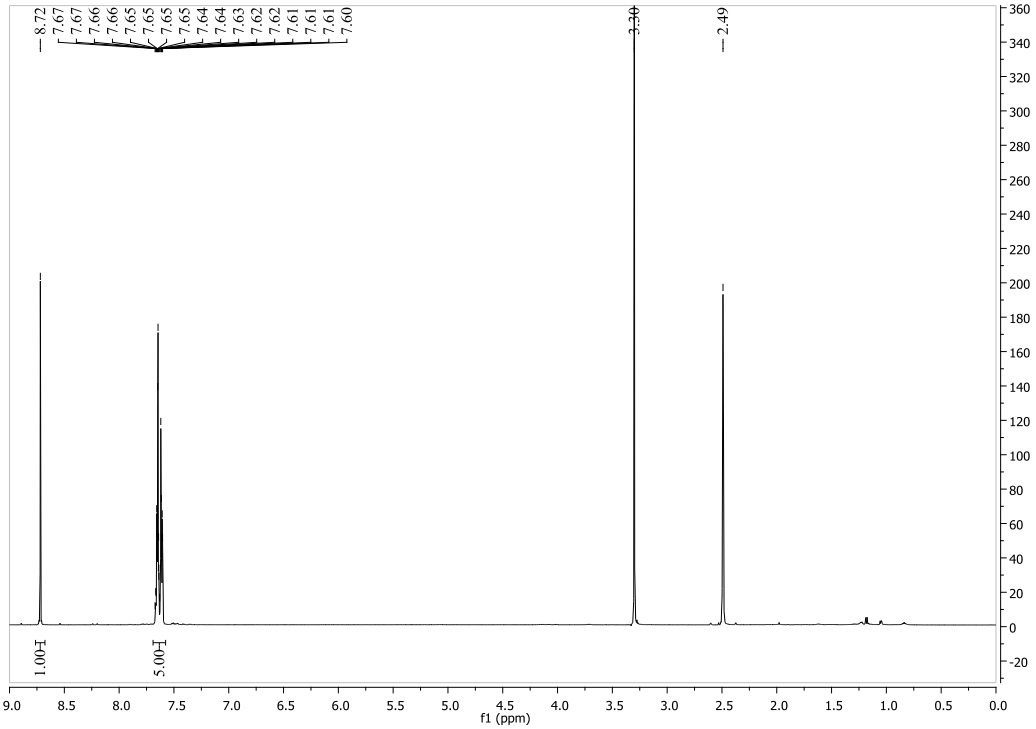
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.72 (s, 1H, H-2), 7.69 – 7.58 (m, 5H, H-11, H-12 and H-13).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 153.9 (C-4 or C-6), 152.2 (C-2), 147.6 (C-4 or C-6), 135.9 (C-8), 133.2, 131.1 (C-5), 130.2 (C-13), 129.6 (C-11 or C-12), 128.2 (C-11 or C-12).

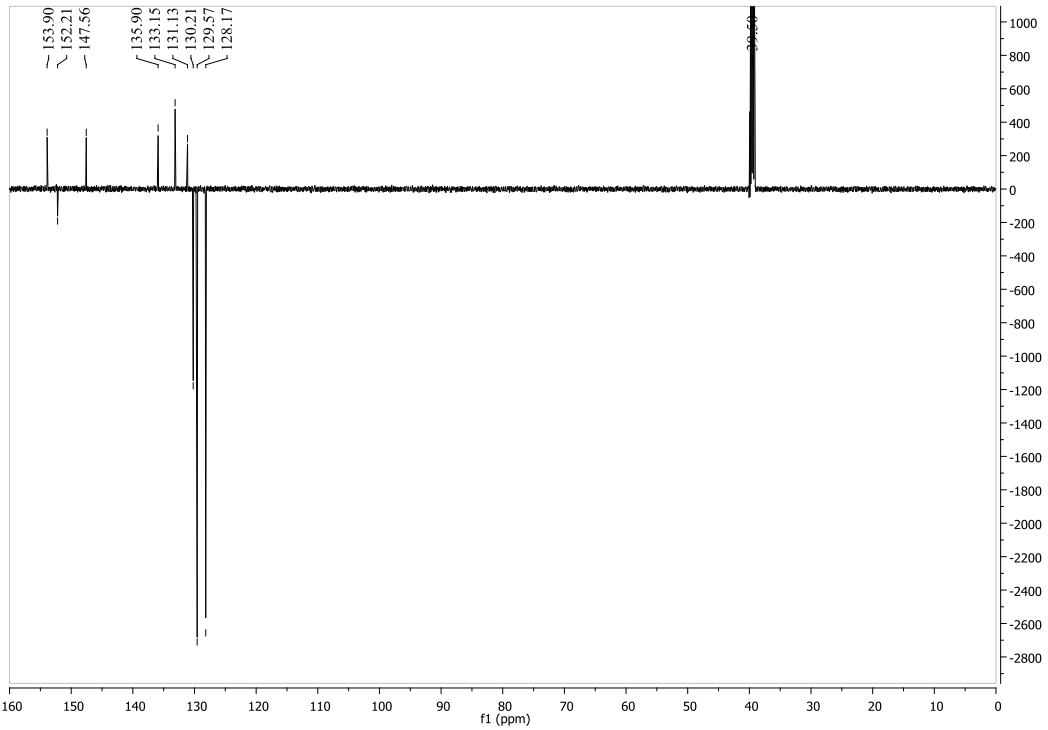
MS EI *m/z* (rel. %) 312/310/308 (24/100/77, M^+), 231/229 (15/45), 194 (13), 167 (14), 114 (6).

HR-MS Found 307.9470, calculated for $C_{11}H_6BrClN_4$ 307.9464.

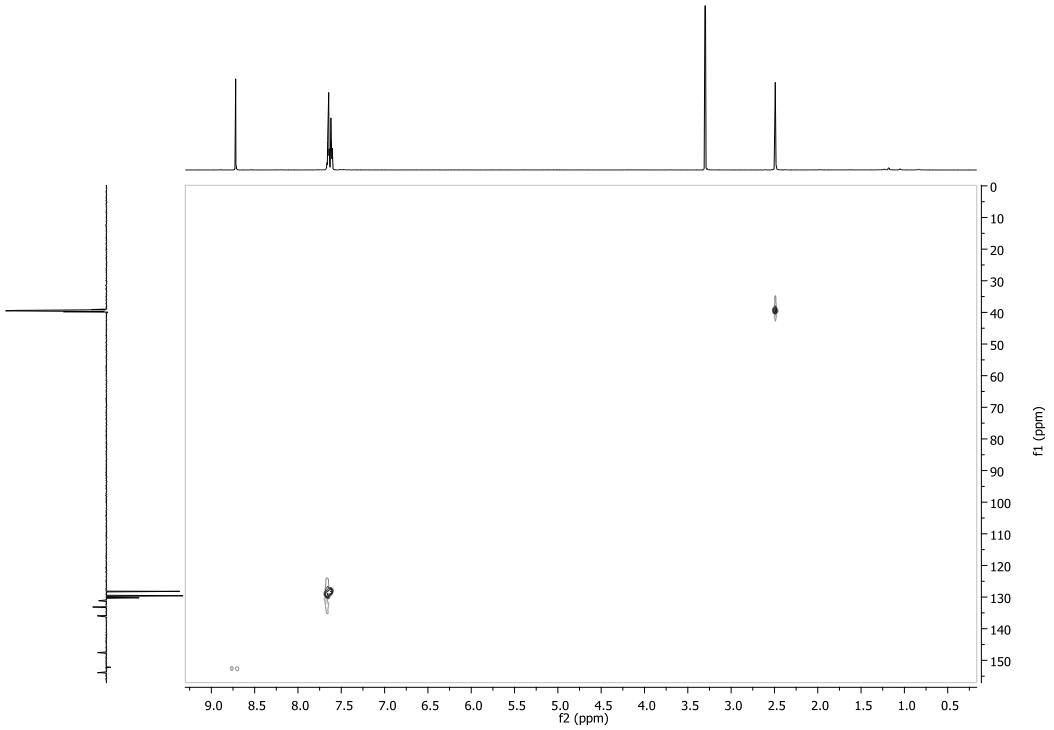
M.p. 234-236 °C.



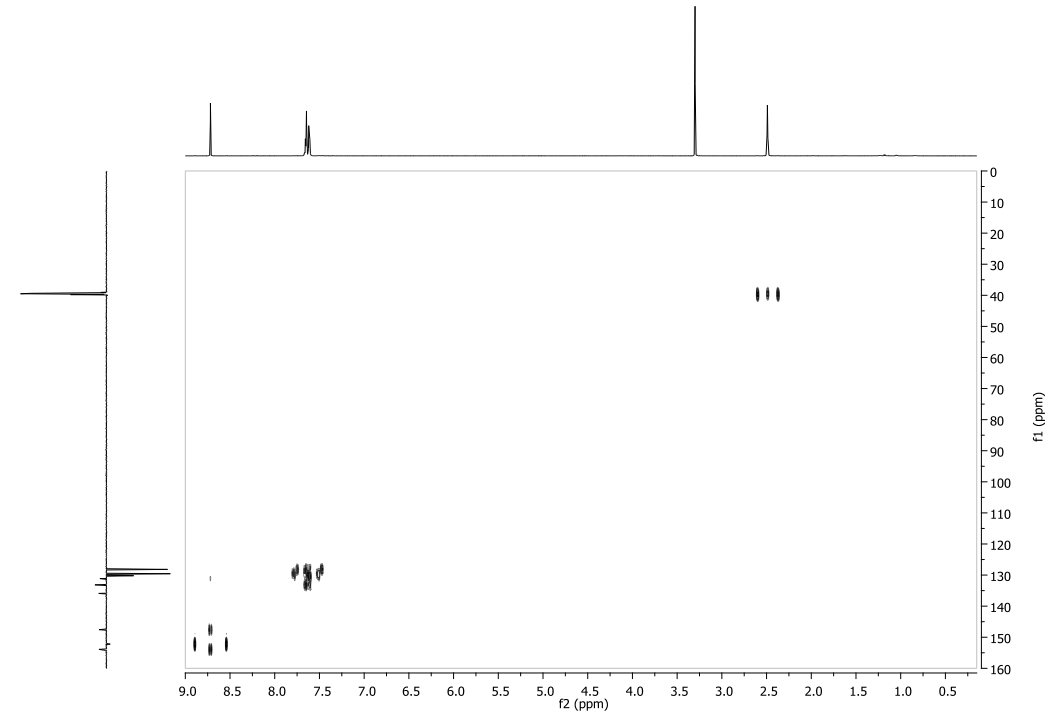
Spectrum 101. ^{1}H NMR of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**44**).



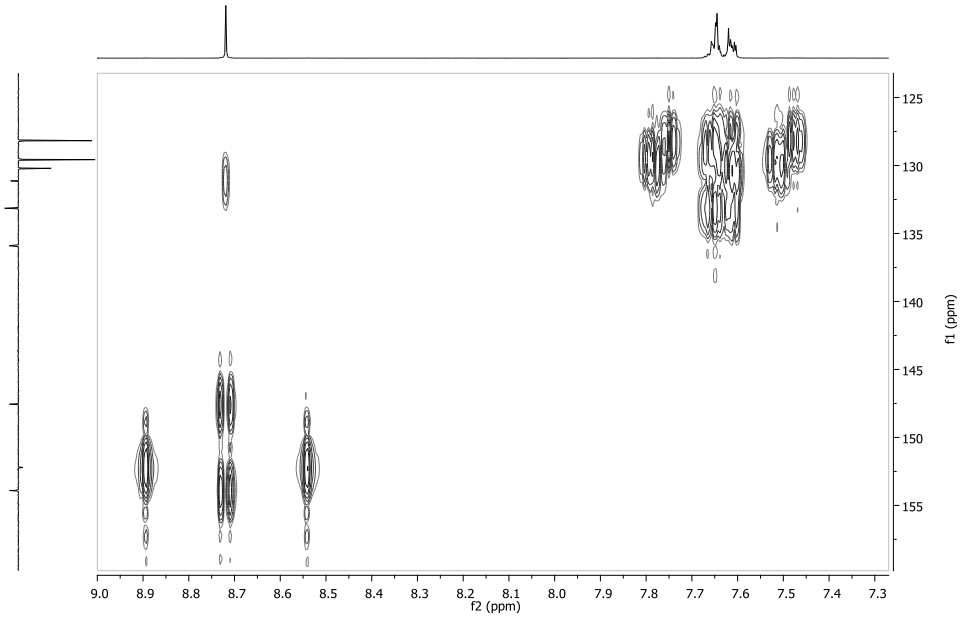
Spectrum 102. ^{13}C NMR of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**44**).



Spectrum 103. HSQC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**44**).



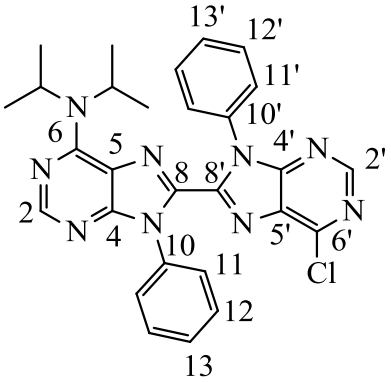
Spectrum 104. HMBC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**44**).



Spectrum 105. HMBC of 8-Chloro-9-phenyl-*9H*-purin-6-amine (**44**), expansion of the aromatic region.

Data for by-product obtained from bromination of 6-chloro-9-phenylpurine (45)

Suggested structure:



45

Possible assignment of data:

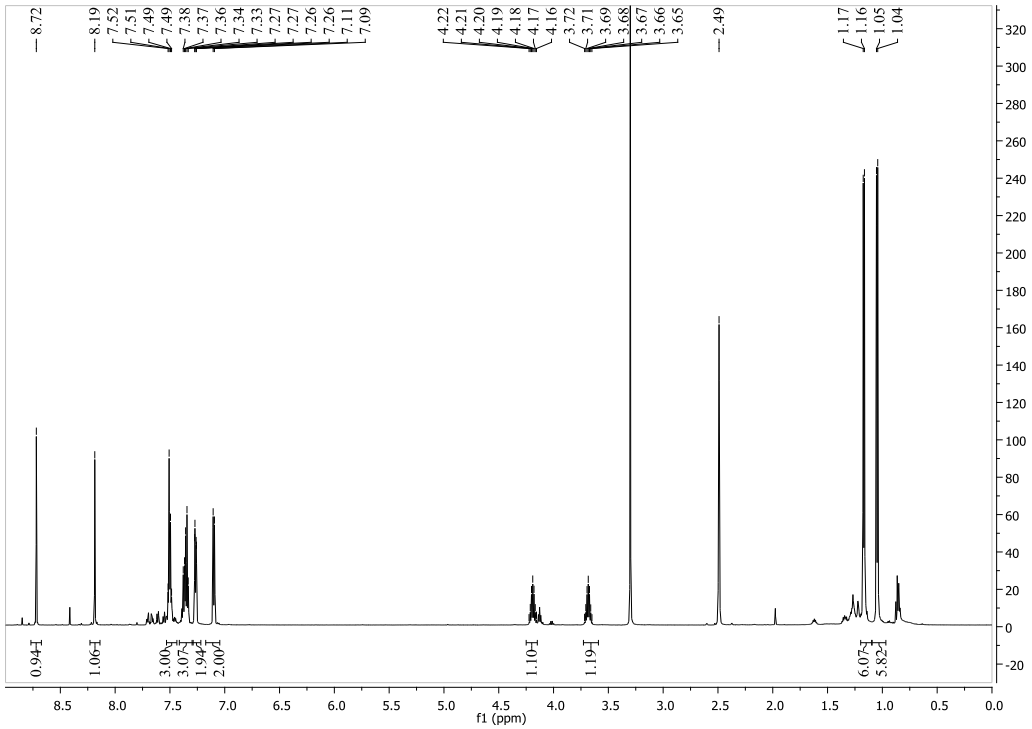
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.72 (s, 1H, 1 x H-2), 8.19 (s, 1H, 1 x H-2), 7.52 – 7.49 (m, 3H, Ph-H), 7.41 – 7.29 (m, 3H, Ph-H), 7.27 (dd, *J* = 7.8, 1.4 Hz, 2H, Ph-H), 7.10 (d, *J* = 7.2 Hz, 2H, Ph-H), 4.19 (dp, *J* = 13.7, 6.8 Hz, 1H, NH, isopropyl group), 3.68 (dp, *J* = 13.4, 6.7 Hz, 1H, 1H, NH from isopropyl group), 1.17 (d, *J* = 6.8 Hz, 6H, 2 x methyl, isopropyl group), 1.05 (d, *J* = 6.8 Hz, 6H, 2 x methyl, isopropyl group).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.13 (C-2), 153.3 (C-4 or C-6), 152.9 (C-4' or C-6'), 152.6 (C-2'), 149.4 (C-4' or C-6'), 144.4*, 134.1 (C-10 or C-10'), 133.5 (C-10 or C-10'), 131.9*, 130.2 (C-5'), 129.1 (C-12 or C-12'), 129.0 (C-13 or C-13'), 128.6 (C-12 or C-12'), 128.4 (C-13 or C-13'), 127.3 (C-11 or C-11'), 127.0 (C-11 or C-11'), 116.9*, 97.2*, 48.2 (CH, isopropyl group), 46.3 (CH, isopropyl group), 22.86 (methyl, isopropyl group), 19.0 (methyl, isopropyl group). * These shifts cannot be assigned from 2D NMR correlations. They could be the missing peaks for C-4 or C-6, C5, C8 and C8' or they could belong to an impurity.

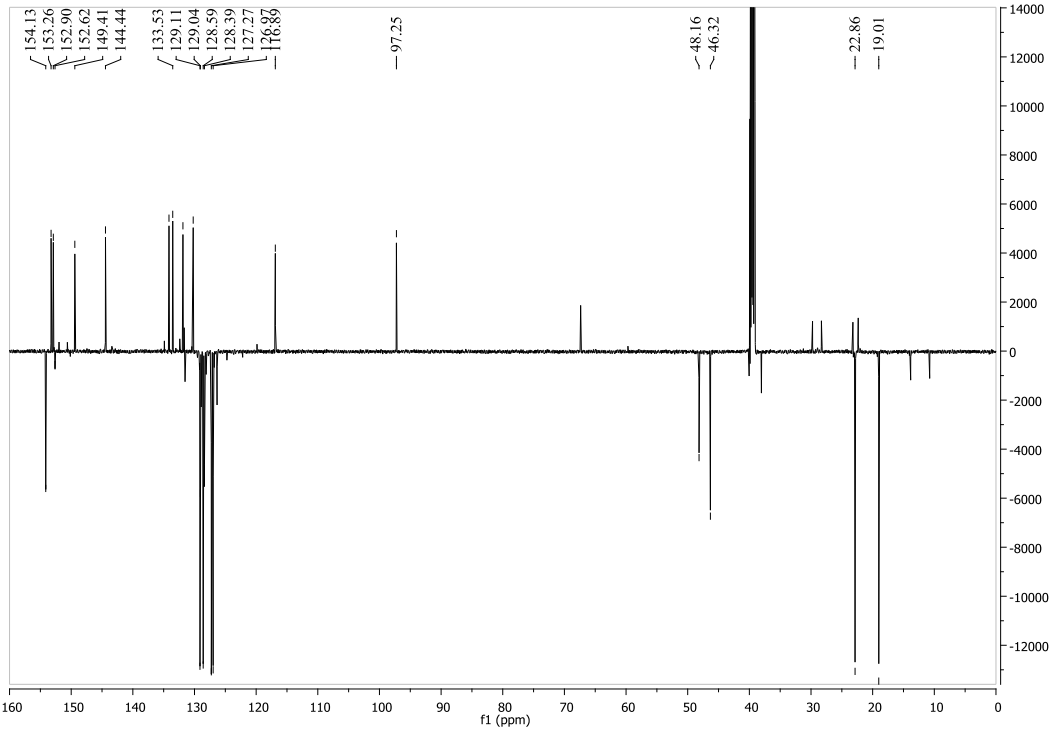
MS EI *m/z* (rel. %) 525/523 (37/100, M^+), 482/480 (31/85), 413 (18), 262 (6), 77 (8).

HR-MS Found 523.1993, calculated for $C_{28}H_{26}ClN_9$ 523.2000.

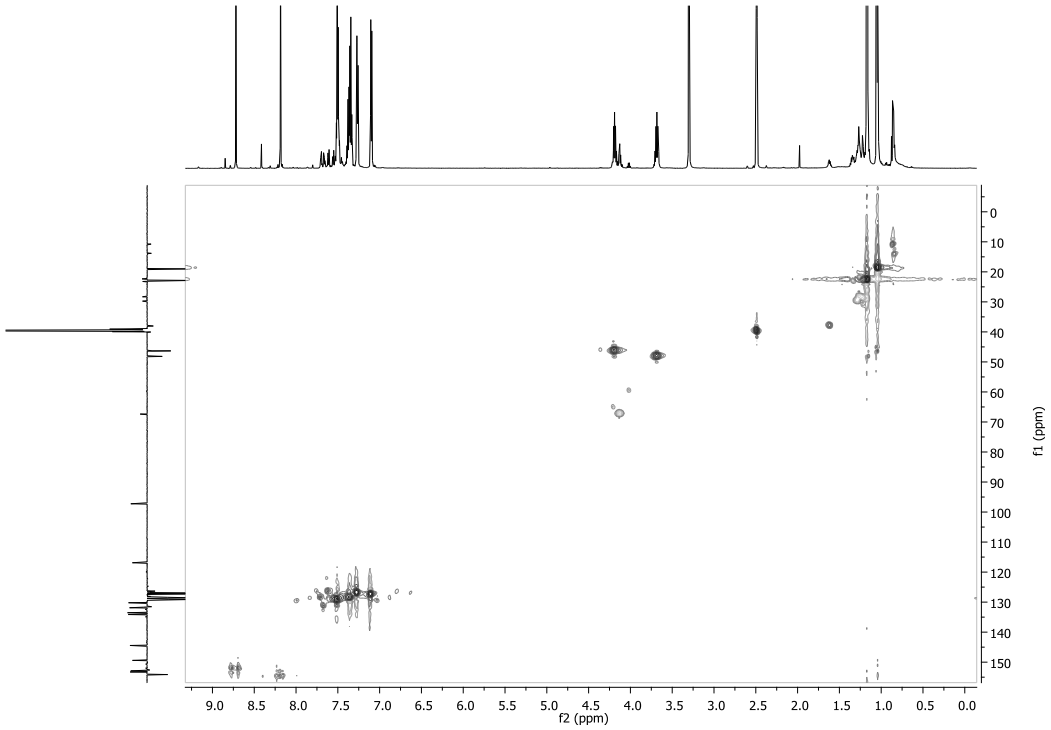
M.p. not obtained.



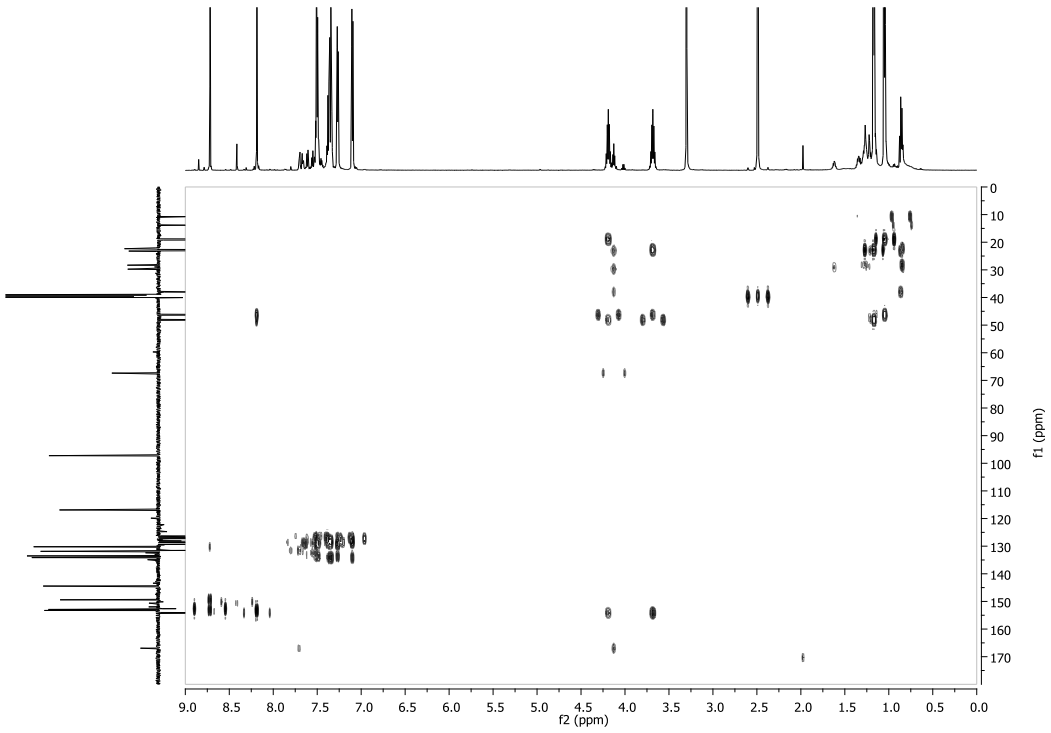
Spectrum 106. ^{1}H NMR of Unknown By-product (**45**).



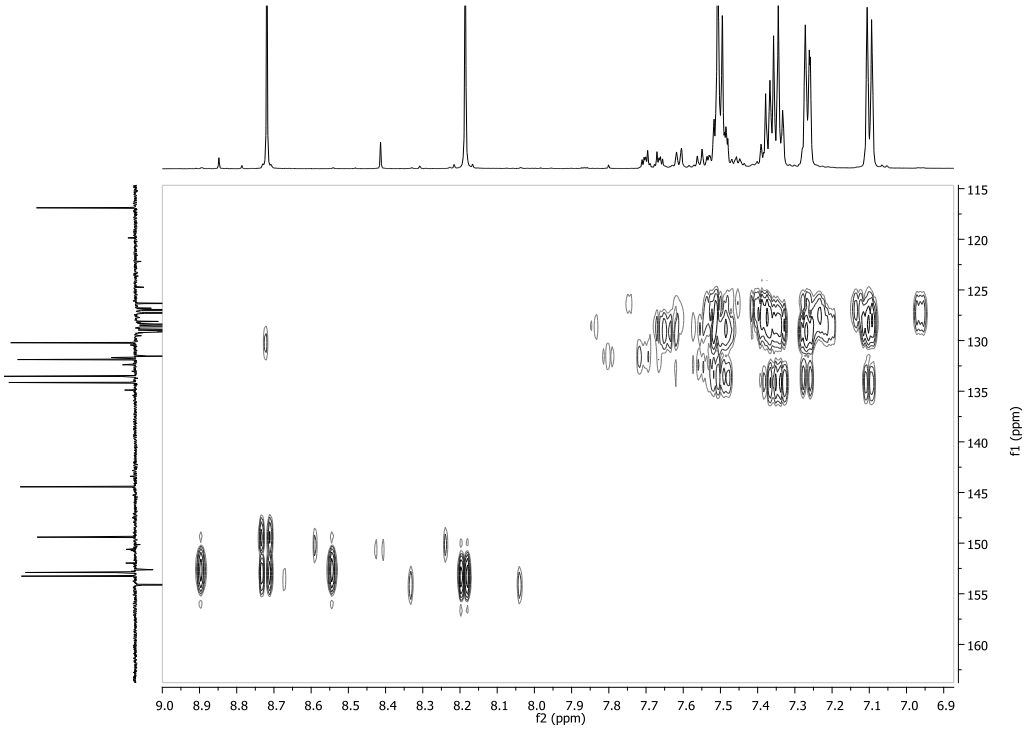
Spectrum 107. ^{13}C NMR of Unknown By-product (**45**).



Spectrum 108. HSQC of Unknown By-product (**45**).

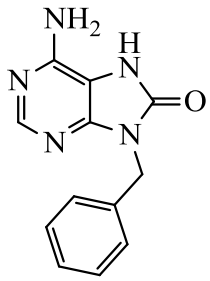


Spectrum 109. HMBC of Unknown By-product (**45**).

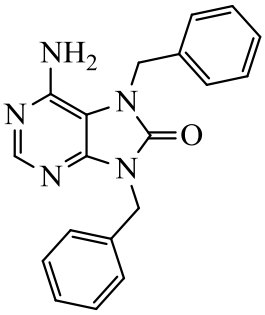


Spectrum 110 HMBC of Unknown By-product (**45**), expansion of the aromatic region.

6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (3a) and 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (39a)



3a

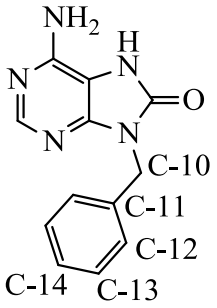


39a

Method 1: 9-Benzyl-8-bromoadenine (**6a**) (63.7 mg, 0.12 mmol) was refluxed in formic acid (5 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 5 mL water and product dried *in vacuo*. The residue was purified by flash chromatography (5-10% methanol in dichloromethane) to give **3a** as a colourless powder (103 mg, 85%).

Method 2: A mixture of 8-oxoadenine (118 mg, 0.781 mmol), DMF (5 mL) and potassium carbonate (218 mg, 1.58 mmol) was stirred at ambient temperature under a nitrogen atmosphere for 15 minutes. Benzyl bromide (0.11 mL, 0.92 mmol) was added and the mixture stirred for another 3 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (5-10% methanol in dichloromethane) to give compounds **3a** (36 mg, 19%) and **39a** (78 mg, ~30%, impure).

6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (3a)



3a

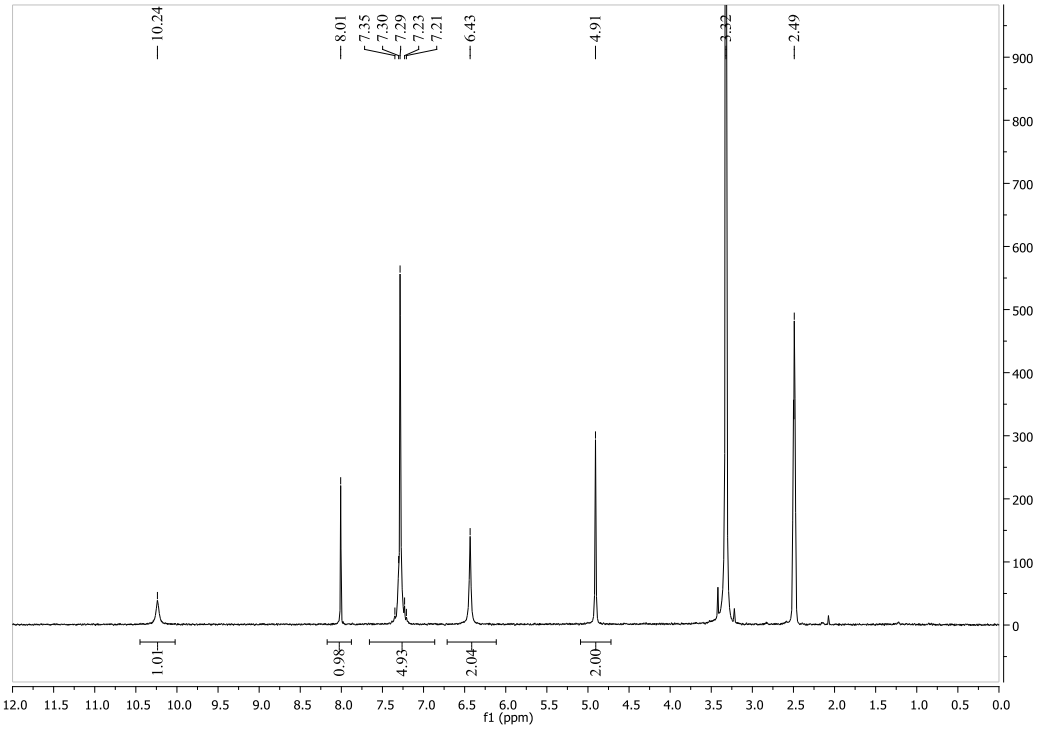
^{1}H NMR (DMSO-d_6, 200 MHz) δ 10.24 (s, 1H, NH), 8.01 (s, 1H, H-2), 7.66 – 6.86 (m, 5H, H-12, H-13 and H-14), 6.43 (s, 2H, NH_2), 4.91 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.0 (C-8), 151.1 (C-2), 147.3 (C-4), 146.67 (C-6), 137.1 (C-11), 128.5 (C-13), 127.39 (C-12), 127.35 (C-14), 103.3 (C-5), 42.39 (C-10).

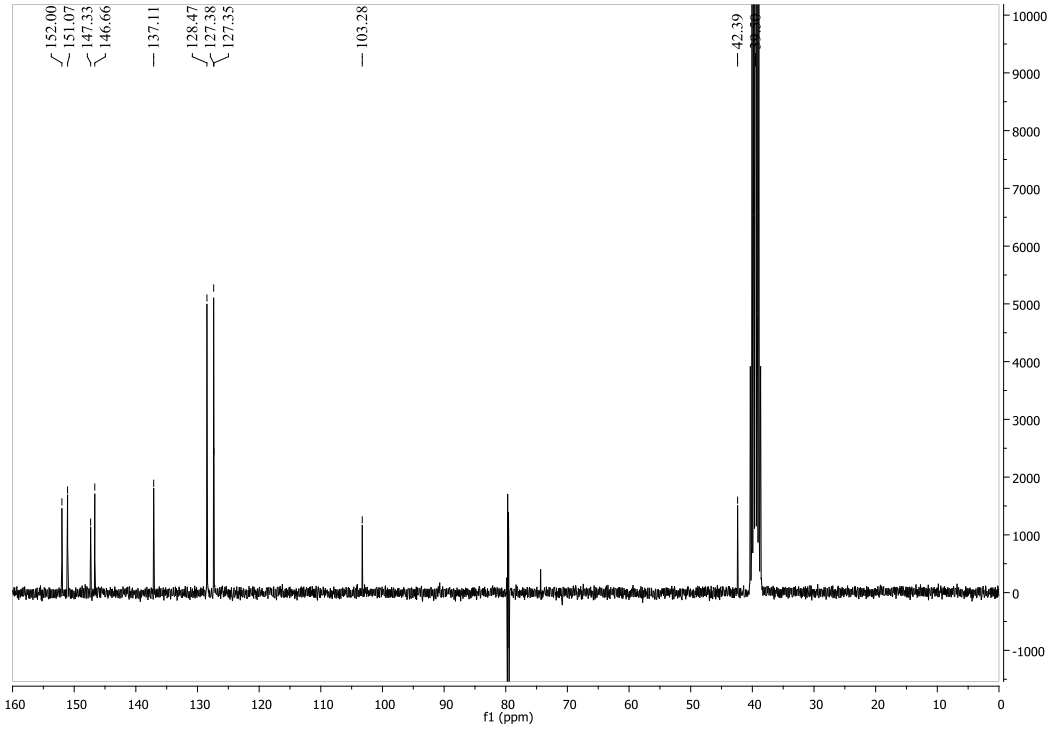
MS EI *m/z* (EI, rel. %) 242 (9), 241 (56, M^+), 240 (13), 225 (5), 212 (13), 136 (19), 110 (6), 91 (100).

HR-MS Found 241.0960, calculated for $C_{12}H_{11}N_5O$ 241.0964.

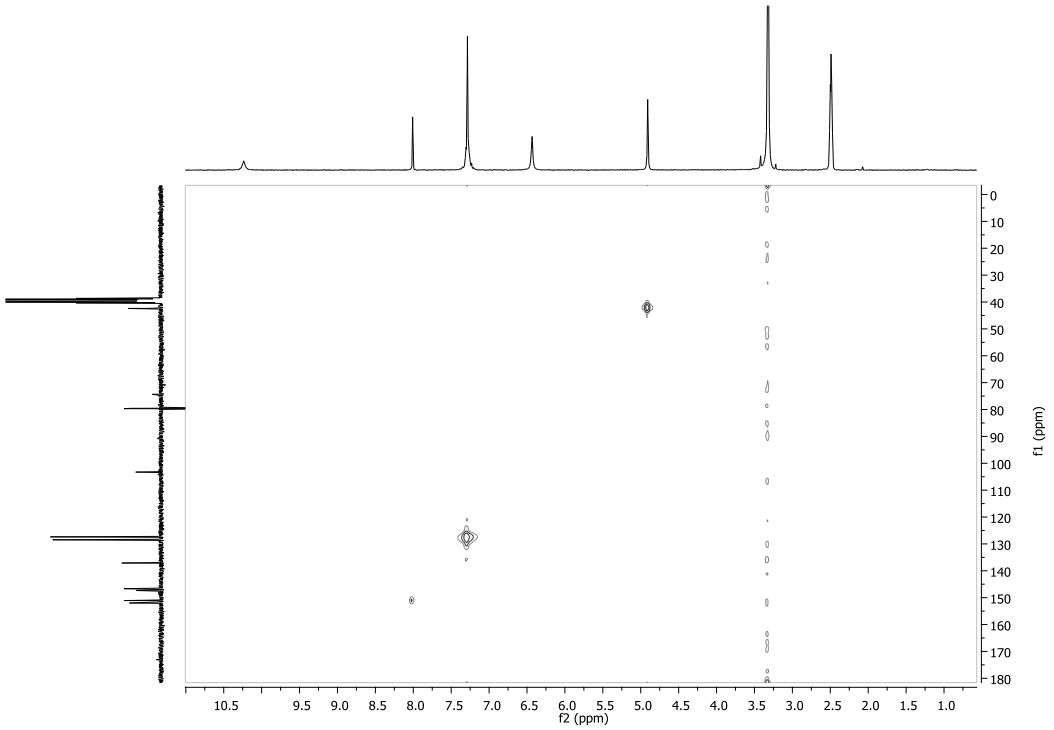
M.p. 265-267 °C.



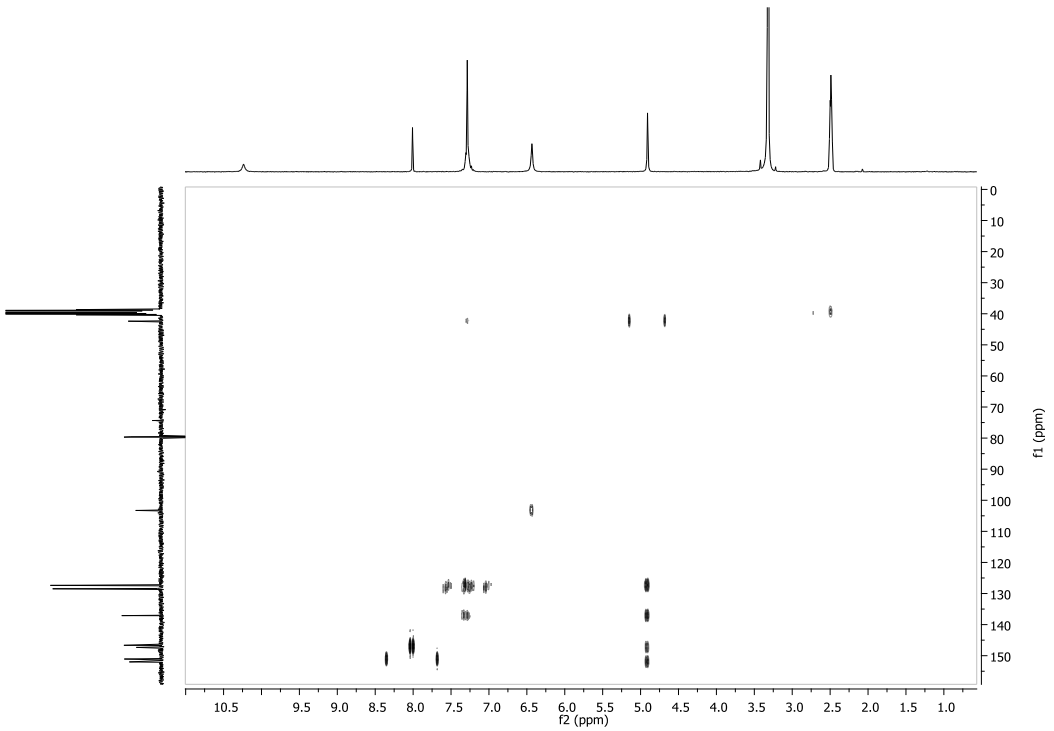
Spectrum 111. ^{1}H NMR of 6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**).



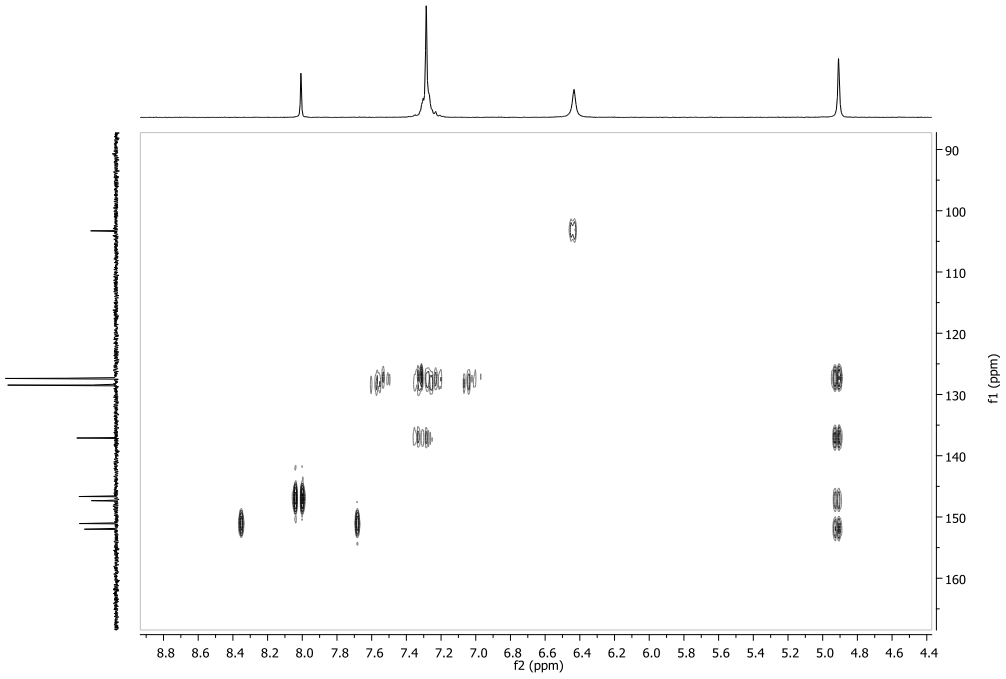
Spectrum 112. ^{13}C NMR of 6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**).



Spectrum 113. HMQC of 6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**).

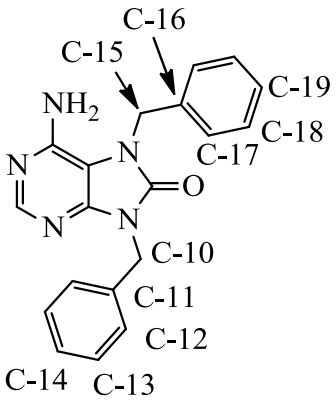


Spectrum 114. HMBC of 6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**).



Spectrum 115. HMBC of 6-Amino-9-benzyl-*7H*-purin-8(*9H*)-one (**3a**), expansion of the aromatic region.

6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (39a)



39a

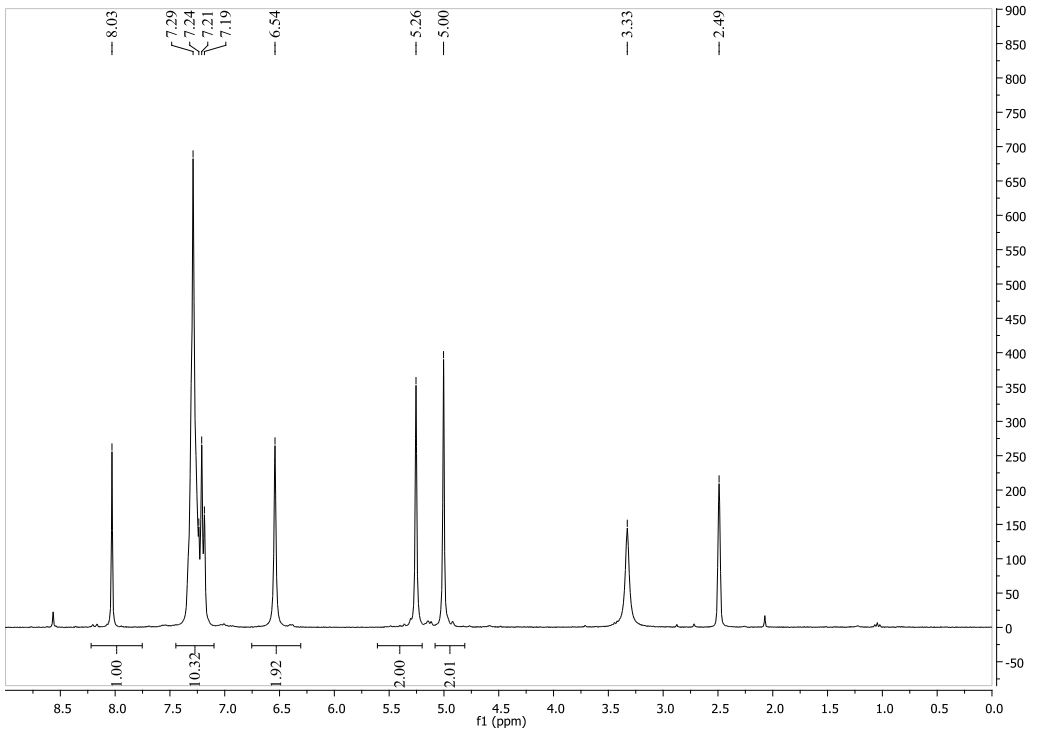
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.03 (s, 1H, H-2), 7.45 – 7.10 (m, 10H, H-12 to H-14 and H-17 to H-19), 6.54 (s, 2H, NH_2), 5.26 (s, 2H, H-15), 5.00 (s, 2H, H-10).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.6 (C-8), 151.1 (C-2), 147.3 (C-4), 147.1 (C-6), 137.8 (C-16), 136.8 (C-11), 128.6, 128.5, 127.41 (C-14 or C-19), 127.39 (C-14 or C-19), 127.3 (C-12), 126.7 (C-17), 104.2 (C-5), 44.2 (C-15), 42.9 (C-10).

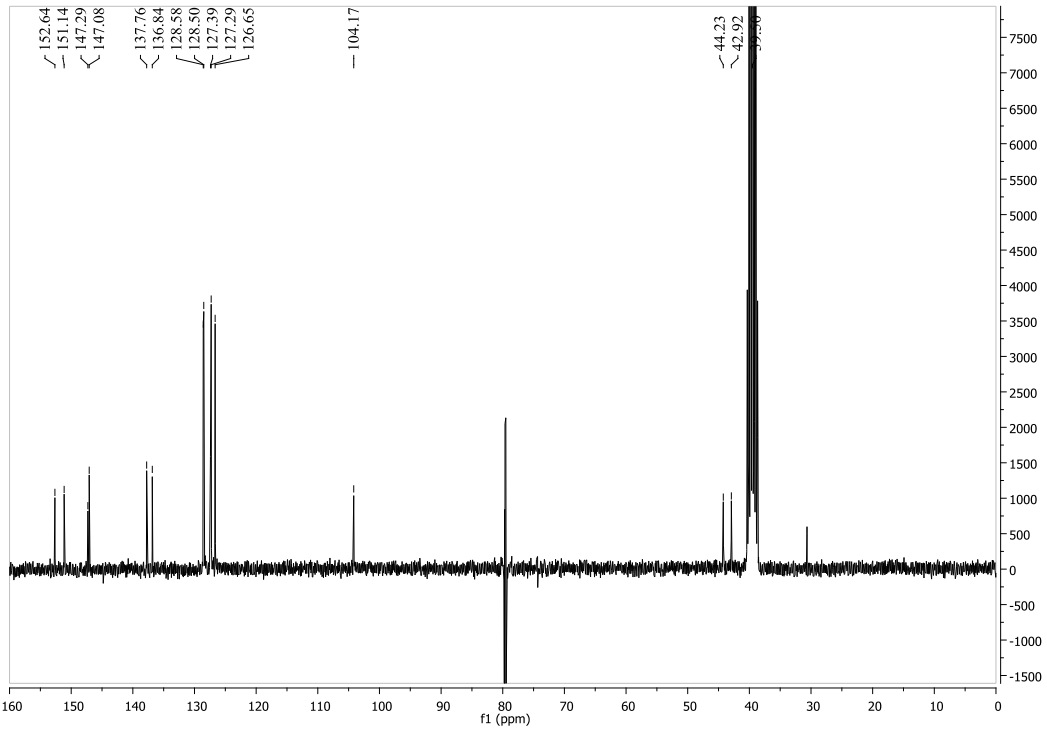
MS EI *m/z* (rel. %) 331 (75, M^+), 240 (22), 91 (100).

HR-MS Found 331.1436, calculated for $C_{19}H_{17}N_5O$ 331.1433.

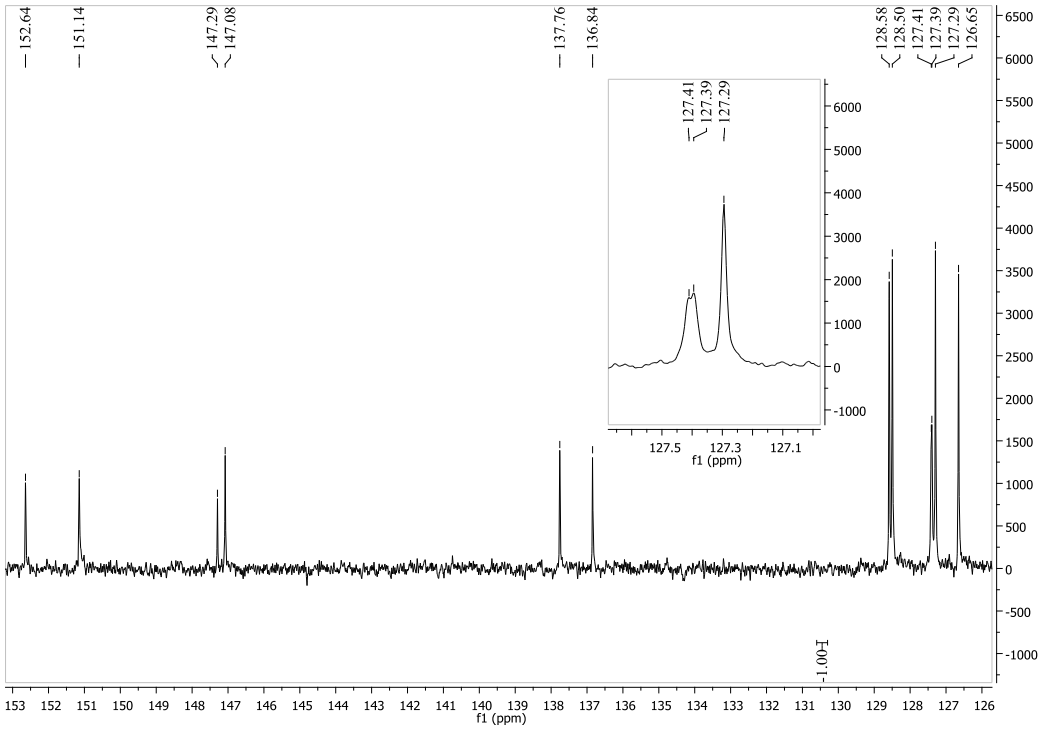
M.p. not obtained.



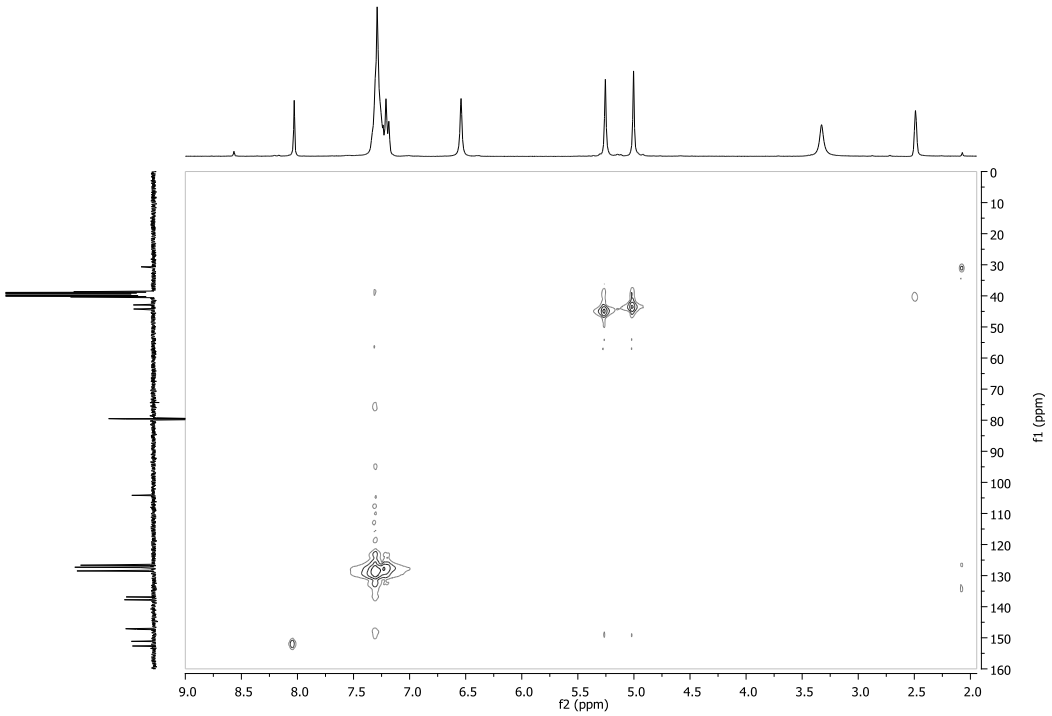
Spectrum 116. ^{1}H NMR of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**).



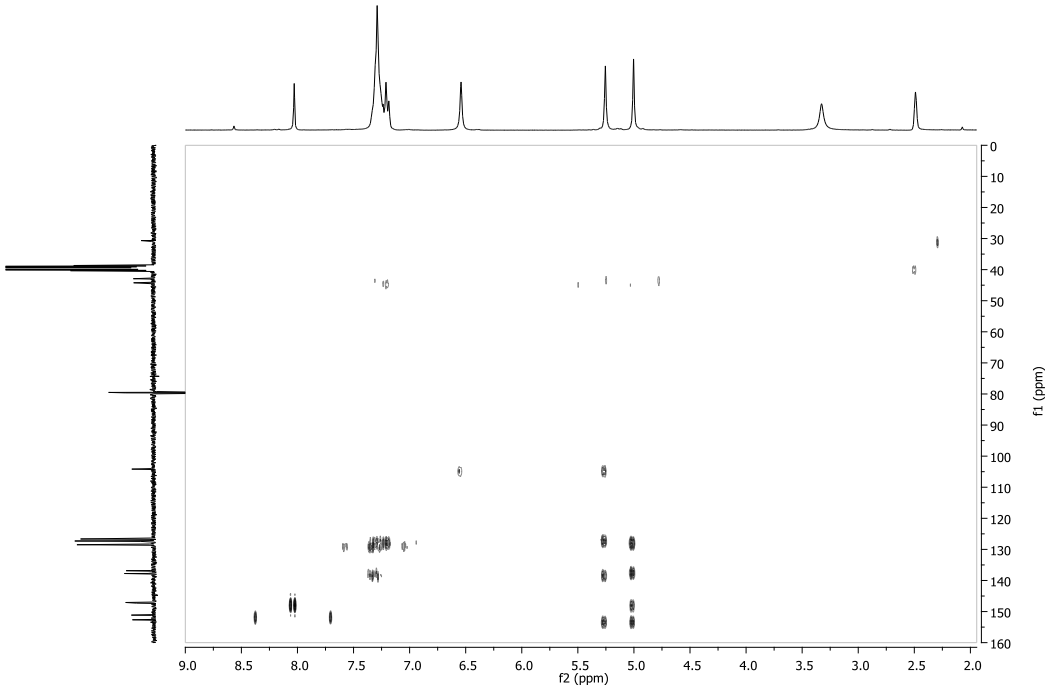
Spectrum 117. ^{13}C NMR of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**).



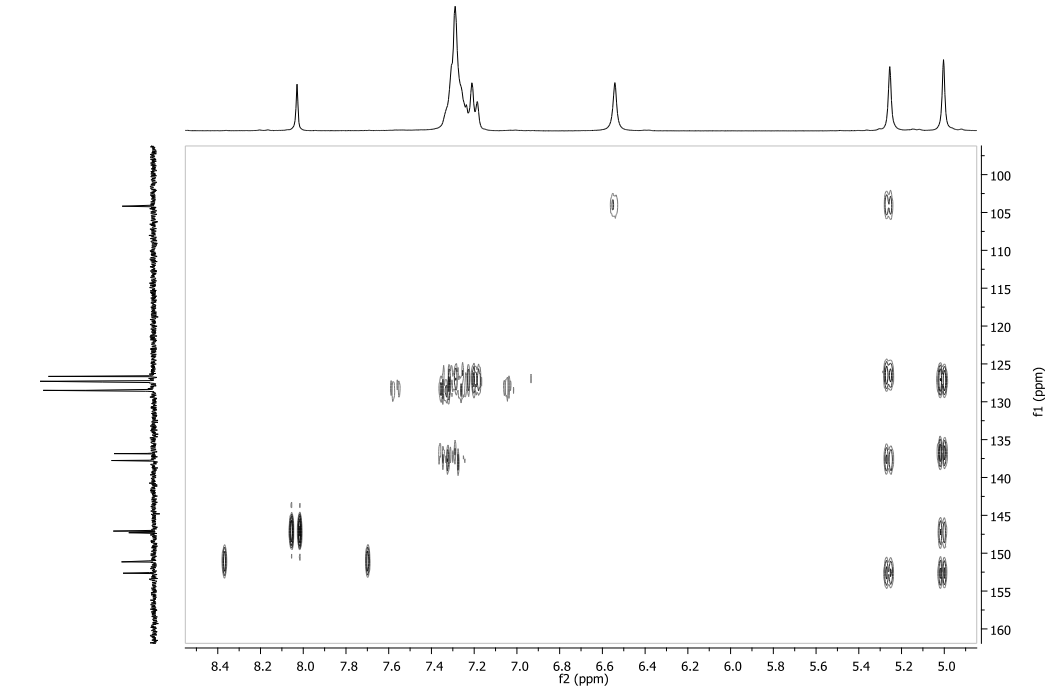
Spectrum 118. ^{13}C NMR of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**), expansion of the aromatic region and part of the phenyl region (inset).



Spectrum 119. HMQC of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**).

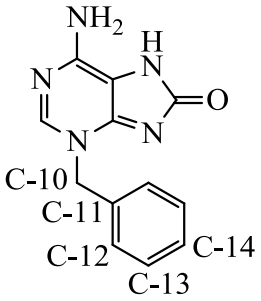


Spectrum 120. HMBC of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**).



Spectrum 121. HMBC of 6-Amino-7,9-dibenzyl-*7H*-purin-8(*9H*)-one (**39a**), expansion of the aromatic region.

6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (38a)



38a

3-Benzyl-8-bromoadenine (**30a**) (152 mg, 0.50 mmol) was refluxed in formic acid (10 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 10 mL water and product dried *in vacuo*. Recrystallized from chloroform and methanol to give **38a** as colourless crystals (40 mg, 33%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.44 (s, 1H, NH), 8.31 (s, 1H, H-2), 7.37-7.31 (m, 5H, H-12, H-13 and H-14), 6.71 (s, 2H, NH_2), 5.30 (s, 2H, H-10).

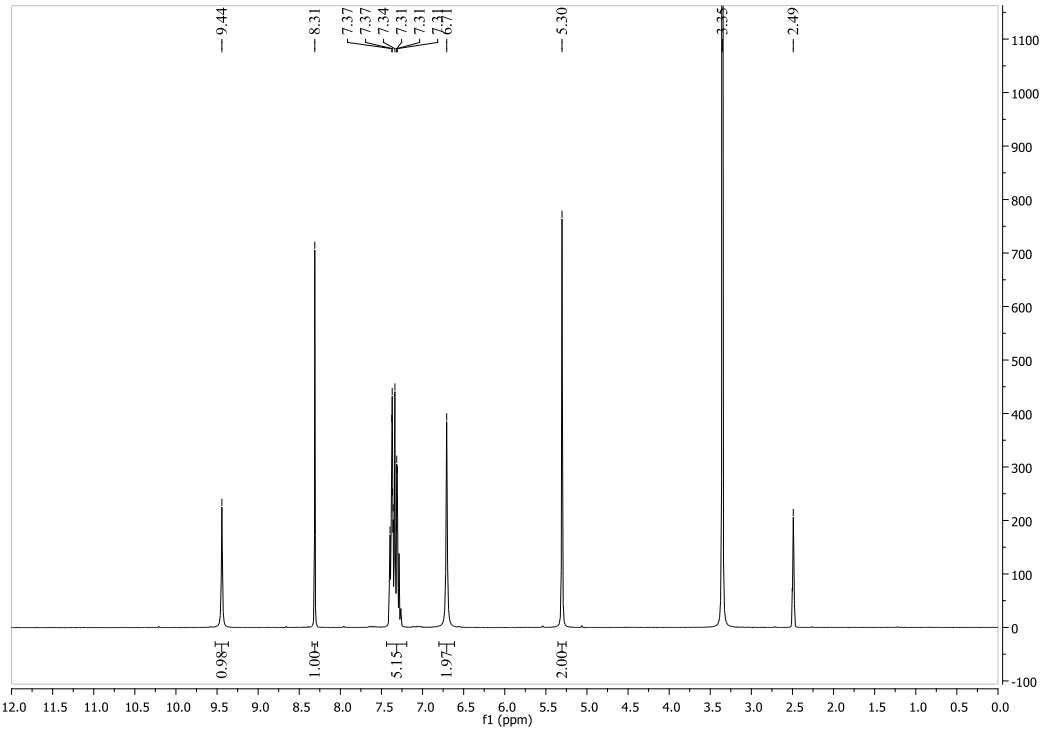
^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.2 (C-8), 153.2 (C-4), 141.8 (C-2), 141.3 (C-6), 136.0 (C-11), 128.6 (C-12 or C-13), 128.0 (C-14), 127.9 (C-12 or C-13), 106.1 (C-5), 51.0 (C-10).

MS EI *m/z* (rel. %) 241 (26, M^+), 240 (10), 91 (100).

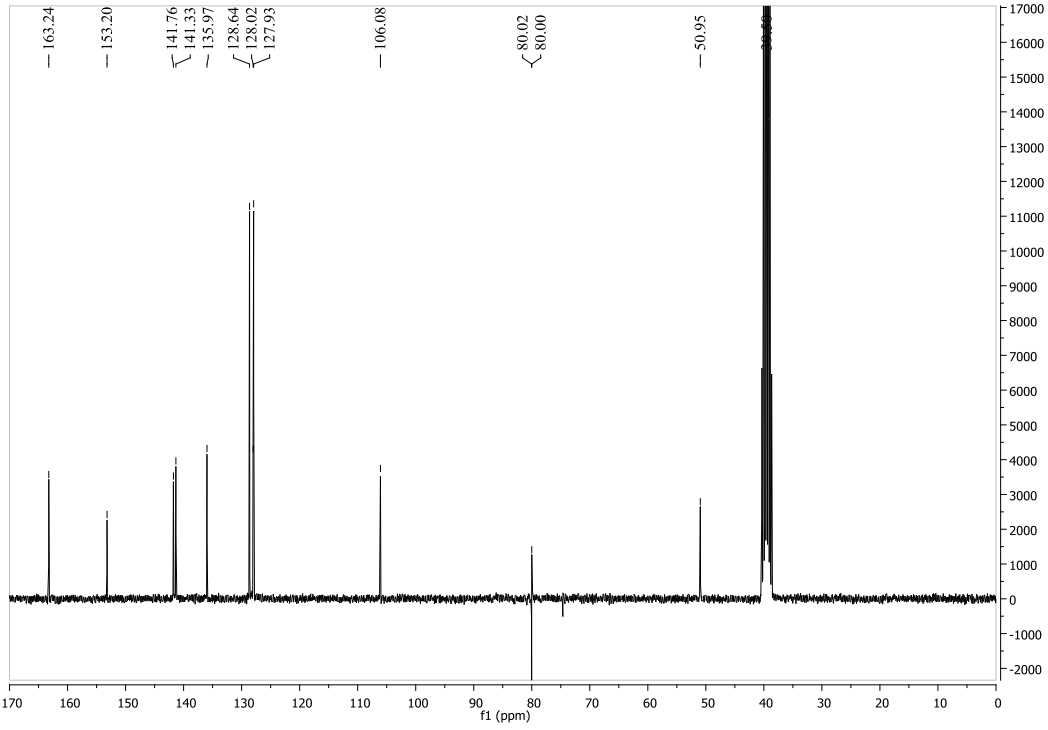
HR-MS Found 241.0955, calculated for $C_{12}H_{11}N_5O$ 241.0964.

Elem. Anal. Found C, 59.93; H, 4.30; N, 28.67. $C_{12}H_{11}N_5O$ requires C, 59.74; H, 4.60; N, 29.03.

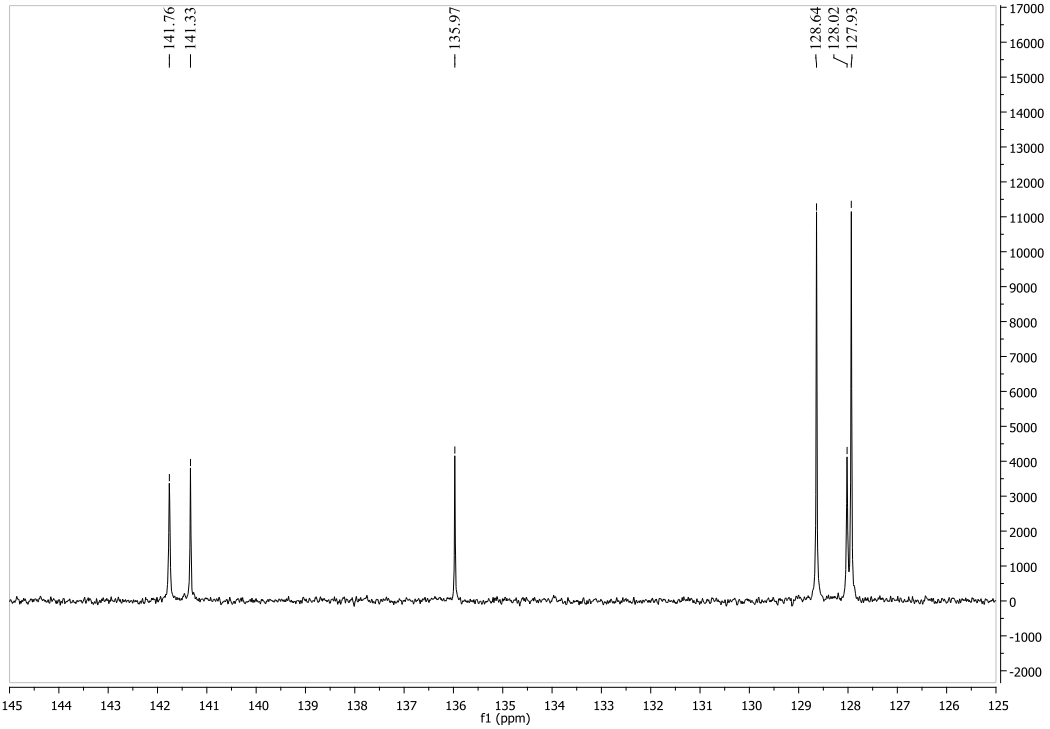
M.p. 228-230 °C.



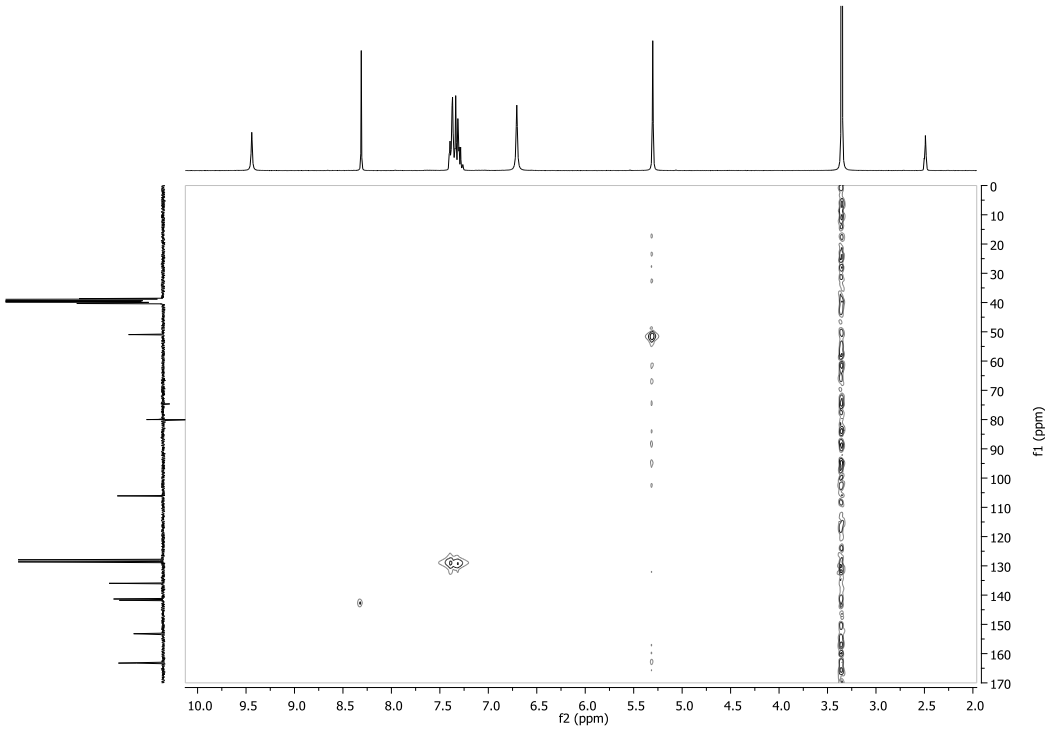
Spectrum 122. ^{1}H NMR of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**).



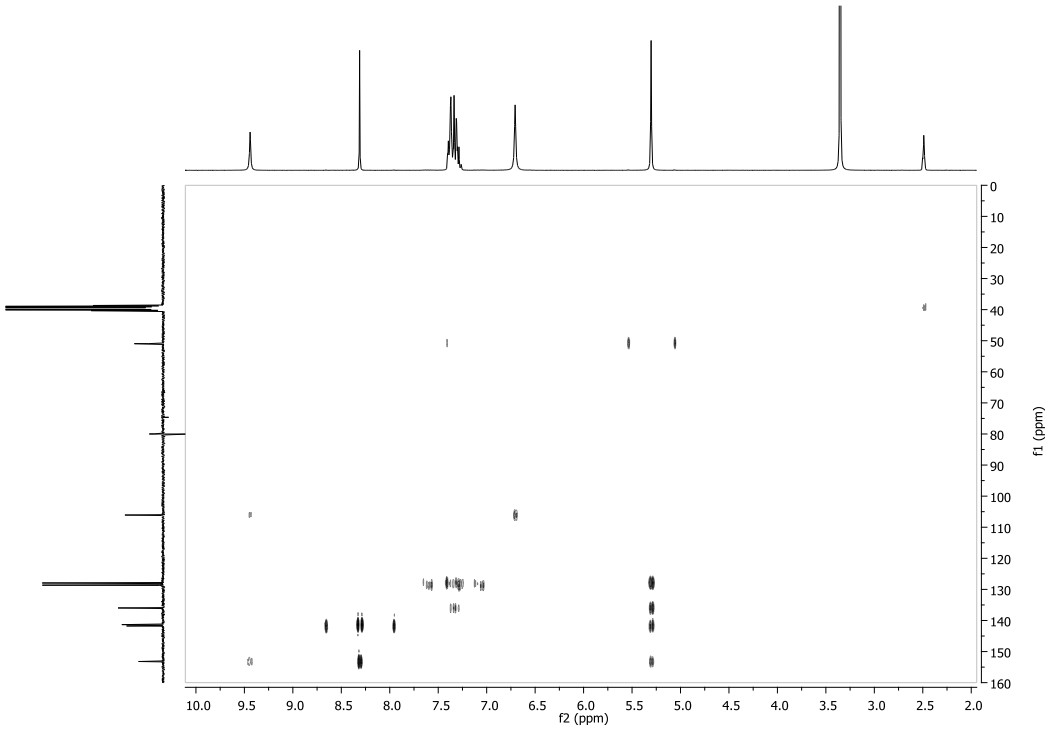
Spectrum 123. ^{13}C NMR of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**).



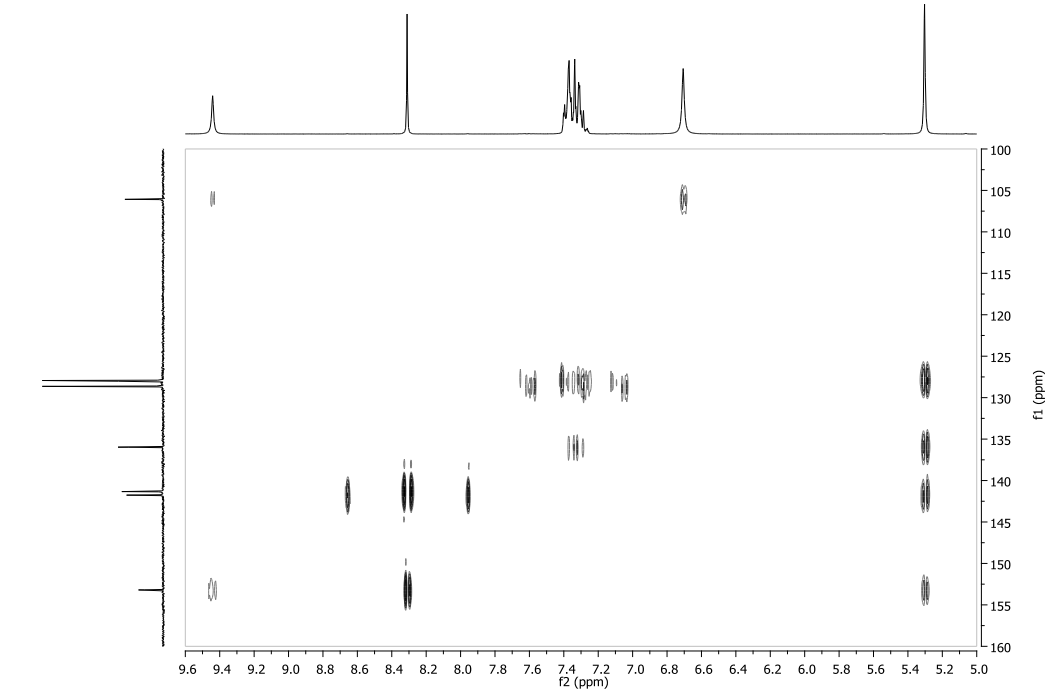
Spectrum 124. ^{13}C NMR of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**), expansion of aromatic and part of purine region.



Spectrum 125. HMQC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**).

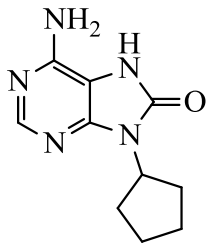


Spectrum 126. HMBC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**).

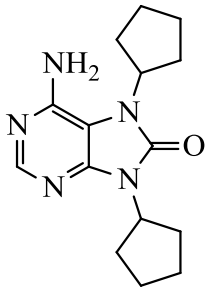


Spectrum 127. HMBC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**38a**), expansion of purine region.

6-Amino-9-cyclopentyl-*7H*-purin-8(*9H*)-one (3b) and 6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (39b)



3b



39b

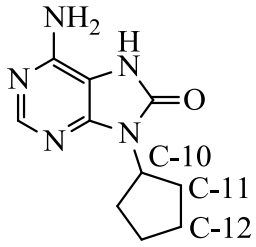
Method 1: 9-Cyclopentyl-8-bromoadenine (**6b**) (237 mg, 0.84 mmol) was refluxed in formic acid (20 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 20 mL water and product dried *in vacuo*. The residue was purified using flash chromatography (0-10% methanol in dichloromethane) to give **3b** as a colourless powder (163 mg, 89%).

Method 2: 9-Cyclopentyl-8-chloroadenine (**8b**) (253 mg, 1.60 mmol) was refluxed in formic acid (25 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 25 mL water and product dried *in vacuo* and the residue was purified as for Method 1 to give **3b** as a colourless powder (214 mg, 92%).

Method 3: A mixture of 8-oxoadenine (**2a**) (121 mg, 0.801 mmol), DMF (5 mL) and potassium carbonate (228 mg, 1.65 mmol) was stirred at 40 °C under a nitrogen atmosphere for

15 minutes. Cyclopentyl bromide (0.10 mL, 0.96 mmol) was added and the mixture stirred for 2 days at that temperature. The temperature was then raised to 60 °C and stirred for another 3 days. The mixture was filtered and the solvent evaporated *in vacuo* and the residue was purified as for Method 1 to give **3b** as a colourless powder (45 mg, 27%) and **39b** (29 mg, ~12%, impure).

6-Amino-9-cyclopentyl-*7H*-purin-8(*9H*)-one (3b)



3b

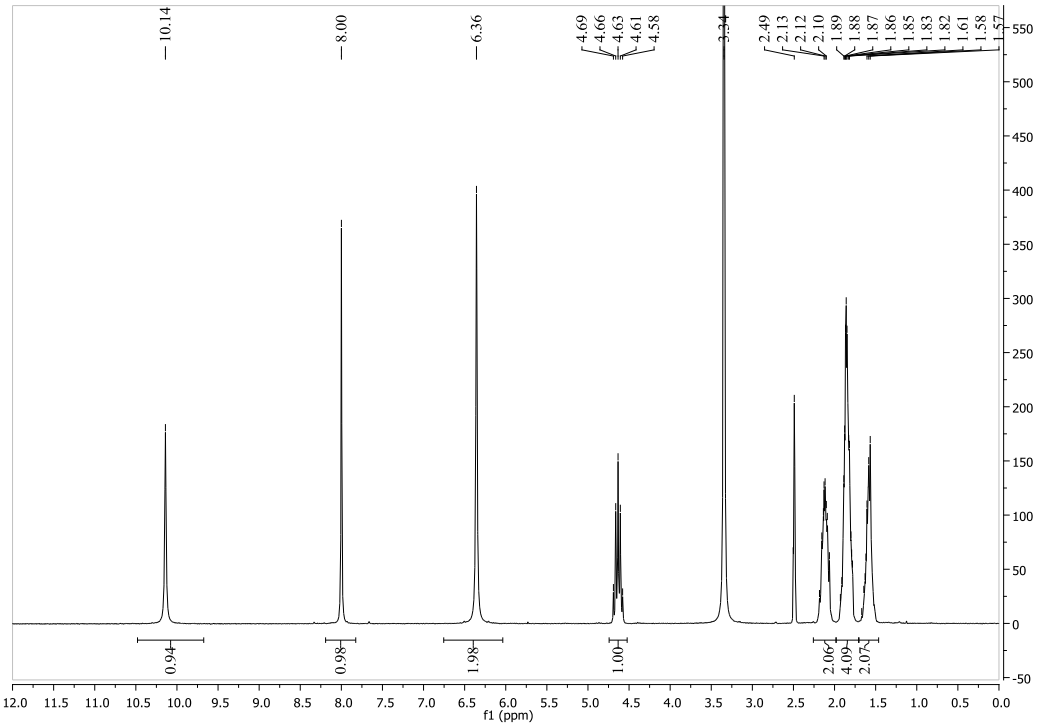
^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.14 (s, 1H, NH), δ 8.00 (s, 1H, H-2), 6.36 (s, 2H, NH_2), 4.63 (quintet, 1H, H-10, J = 12.3 Hz), 2.18 – 2.01 (m, 2H, C-11 and C-12), 1.90 – 1.81 (m, 4H, C-11 and C-12), 1.63 – 1.60 (m, 2H, C-11 and C-12).

^{13}C NMR (DMSO-d_6, 100 MHz) δ 151.7 (C-8), 150.6 (C-2), 147.3 (C-4), 146.6 (C-6), 103.2 (C-5), 52.0 (C-10), 29.0 (C-11), 24.3 (C-12).

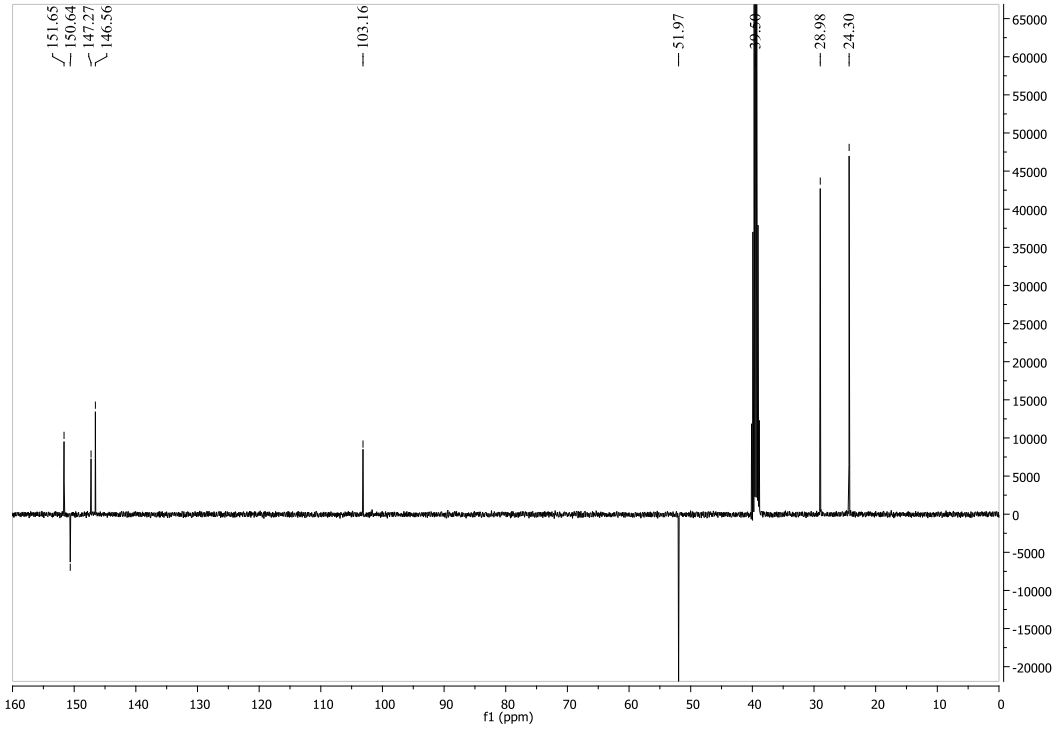
MS EI *m/z* (rel. %) 219 (21, M^+), 178 (9), 151 (100), 124 (6).

HR-MS Found 219.1125, calculated for $C_{10}H_{13}N_5O$ 219.1120.

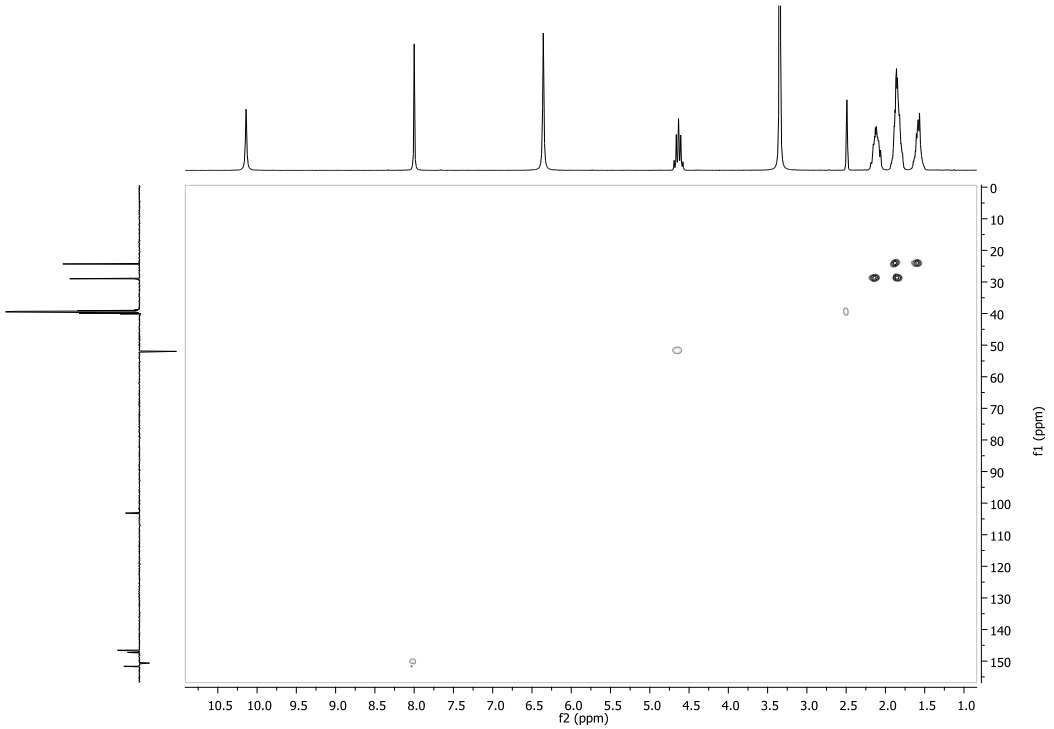
M.p. 220-223 °C.



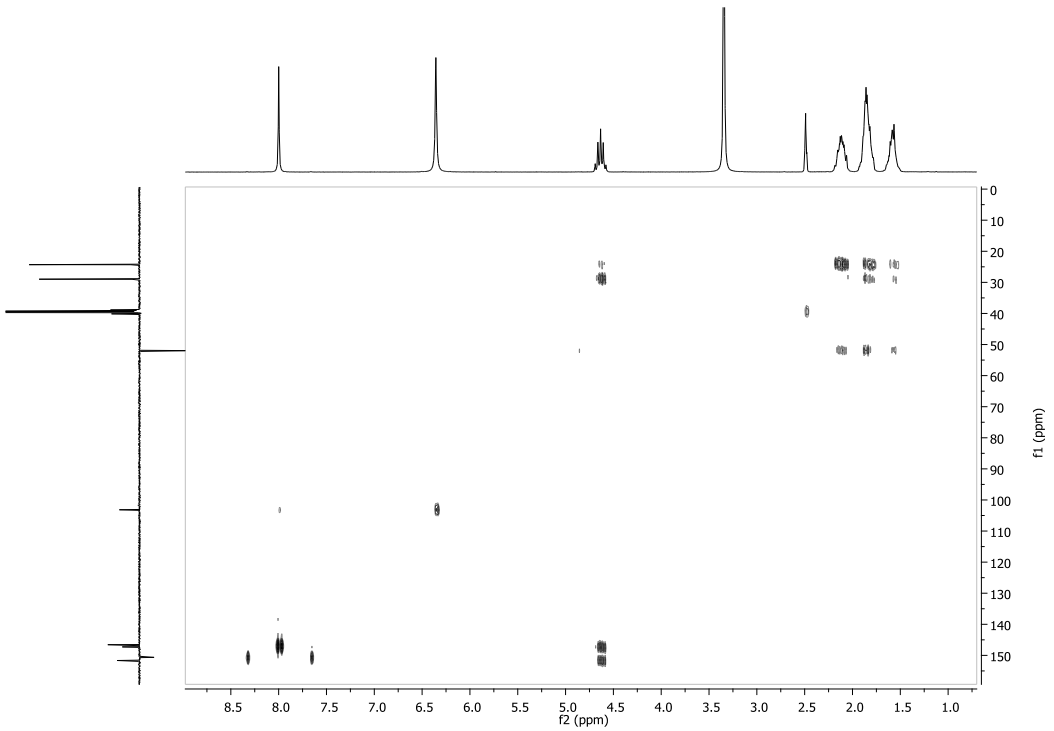
Spectrum 128. ^{1}H NMR of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**3b**).



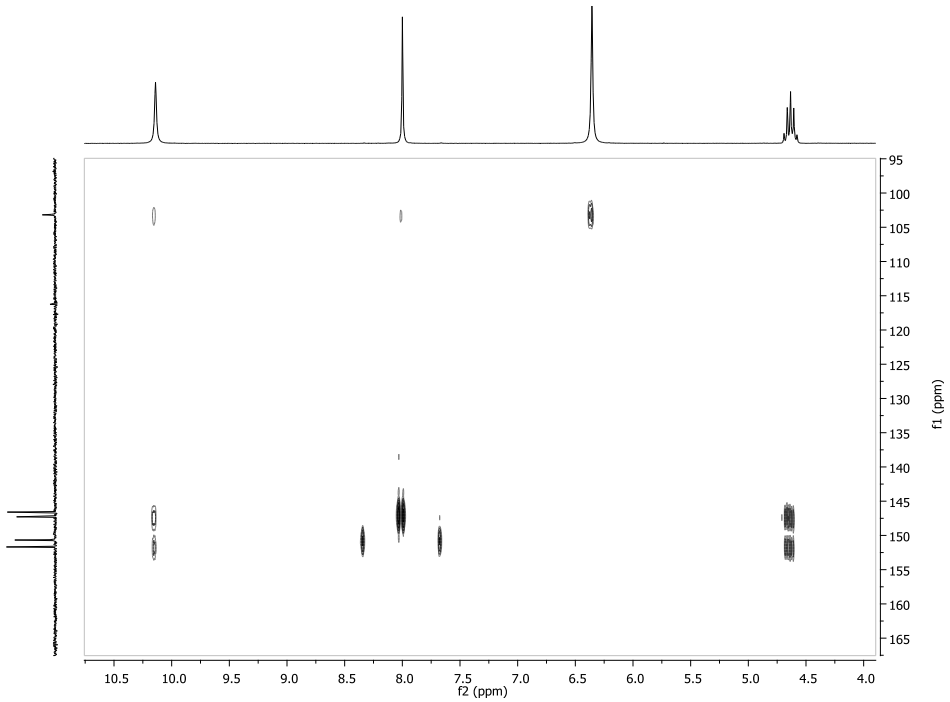
Spectrum 129. ^{13}C APT NMR of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**3b**).



Spectrum 130. HMQC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**3b**).



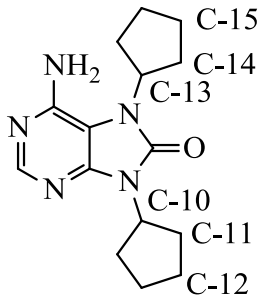
Spectrum 131. HMBC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**3b**).



Spectrum 132. HMBC of 6-Amino-3-benzyl-*3H*-purin-8(*7H*)-one (**3b**), expansion of the aromatic region.

6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (39b)

Suggested structure:*



39b

^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.00 (s, 1H, H-2), 7.20 (s, 10H), 6.69 (s, 2H, NH_2), 5.50 – 5.42 (m, 1H, H-13), 4.94 – 4.62 (m, 1H, H-10), 2.37 – 1.31 (m, 16H, H-11, H-12, H-14 and H-15).

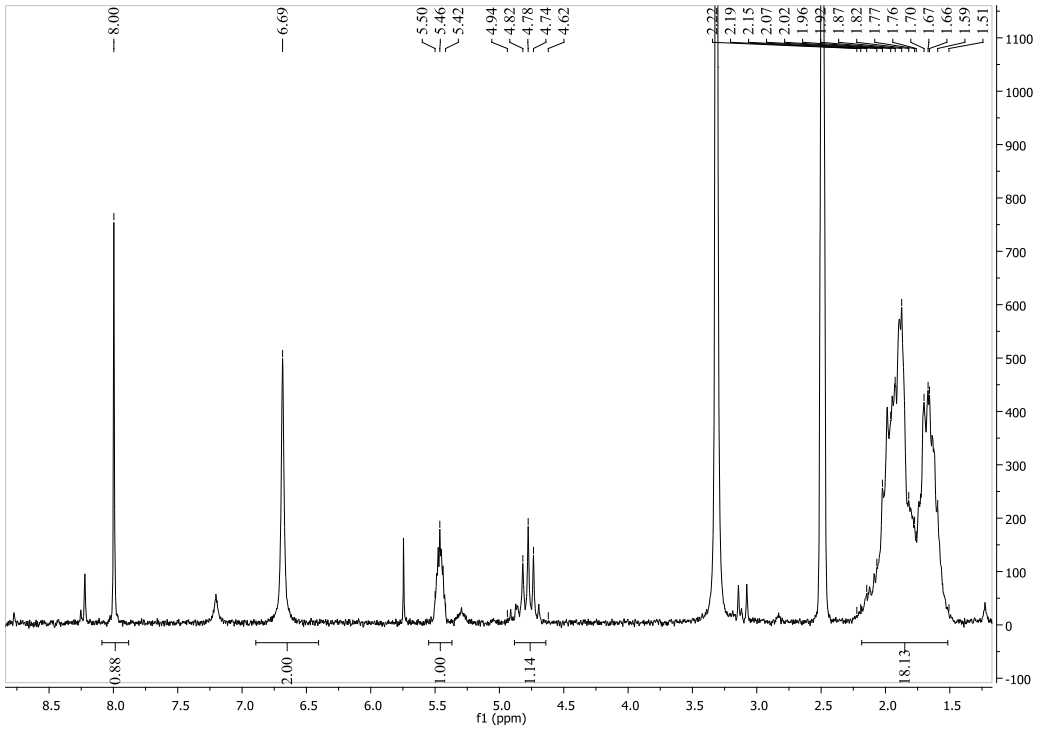
^{13}C NMR (DMSO-d_6, 100 MHz) δ 153.5 (C-6 or C-8), 153.4 (C-6 or C-8), 150.3 (C-2), 149.1 (C-4), 114.7 (C-5), 82.3 (C-13), 53.0 (C-10), 32.3, 30.1, 24.6 and 23.1 (C-11, C-12, C-14 and C-15).

MS EI *m/z* (rel. %) 287 (13, M^+), 219 (35), 190 (5), 178 (9), 151 (100), 124 (5).

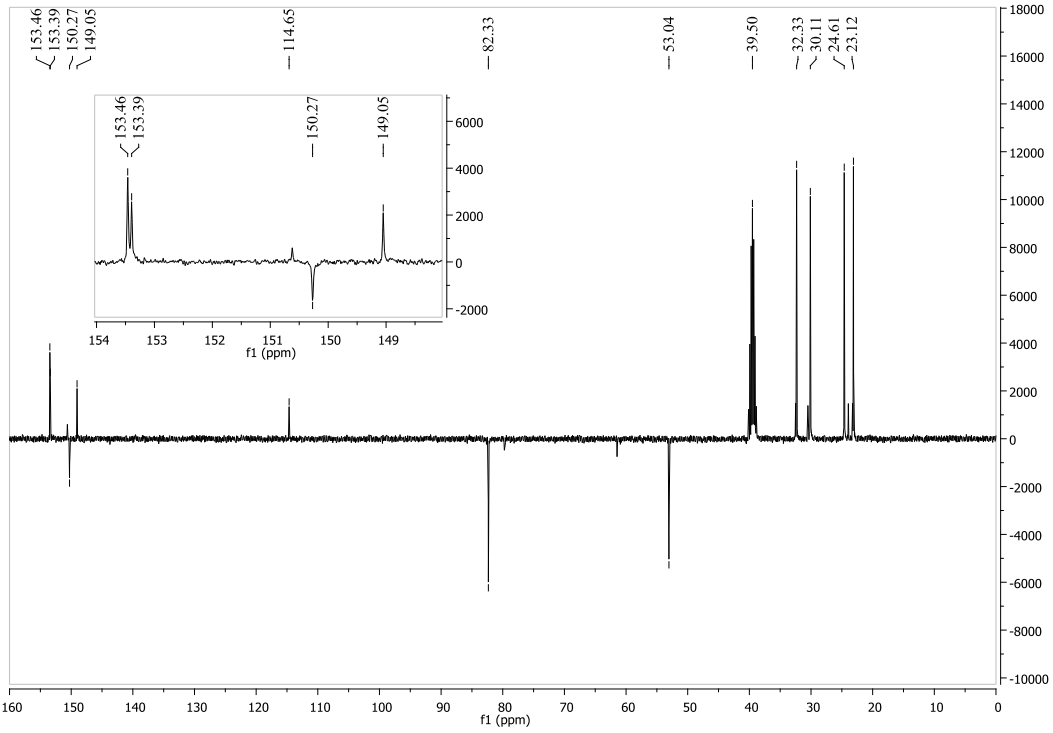
HR-MS Found 287.1751, calculated for $C_{15}H_{21}N_5O$ 287.1746.

M.p. not obtained.

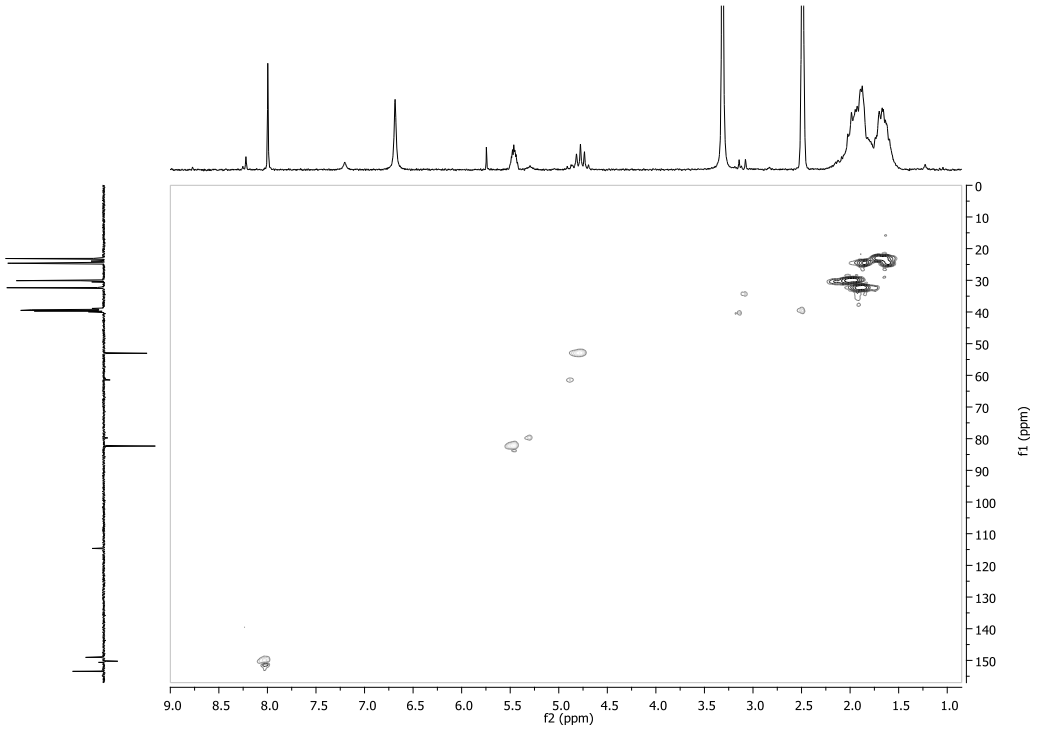
* This structure is suggested partially on the basis that the by-product of these analogous reactions should be similar. It is possible, however, that the cyclopentyl group on *N*-7 is actually on *N*-1 or *N*-3, since the CH on the second side-chain does not show any correlations. The MS data for this compound is in accordance with a dialkylated product of this nature.



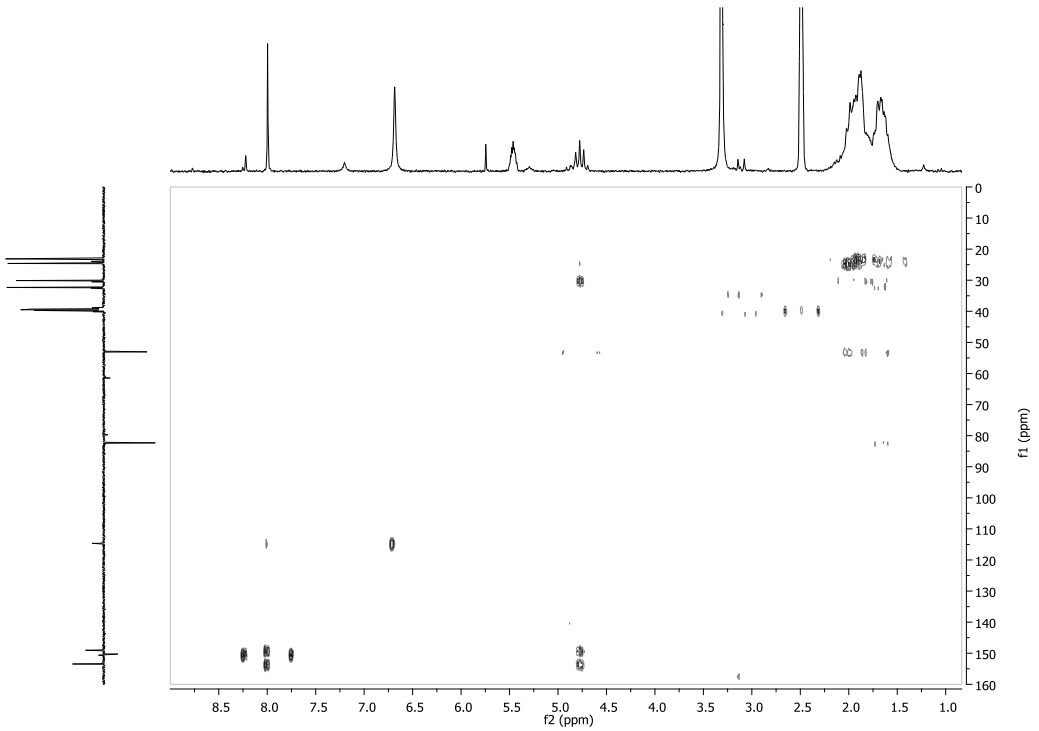
Spectrum 133. [1]H NMR of 6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (**39b**).



Spectrum 134. [13]C APT NMR of 6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (**39b**), with expansion of the purine region.

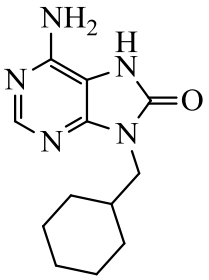


Spectrum 135. HSQC of 6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (**39b**).

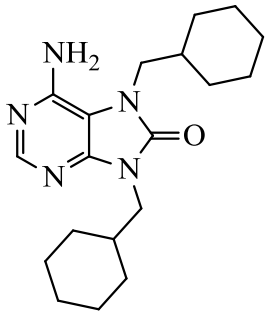


Spectrum 136. HMBC of 6-Amino-7,9-dicyclopentyl-*7H*-purin-8(*9H*)-one (**39b**).

6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (3c) and 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (39c)



3c



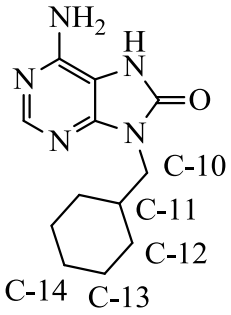
39c

Method 1: 9-(Cyclohexylmethyl)-8-bromoadenine (**6c**) (129 mg, 0.42 mmol) was refluxed in formic acid (10 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 20 mL water and product dried *in vacuo*. The residue was purified using flash chromatography (3-5% methanol in dichloromethane) to give **3c** as a colourless powder (199 mg, 78%).

Method 2: 9-(Cyclohexylmethyl)-8-chloroadenine (**8c**) (180 mg, 0.678 mmol) was refluxed in formic acid (20 mL) overnight. The formic acid was evaporated then co-evaporated with 3 x 20 mL water and product dried *in vacuo*. The residue was purified using flash chromatography (0-10% methanol in dichloromethane) to give **3c** as a colourless powder (151mg, 90%) and an unknown by-product (**48c**) as a colourless powder (7 mg, 4%).

Method 3: A mixture of 8-oxoadenine (**2a**) (128 mg, 0.847 mmol), DMF (5 mL) and potassium carbonate (238 mg, 1.73 mmol) was heated to 50 °C under nitrogen atmosphere. (Bromomethyl)cyclohexane (0.14 mL, 1.013 mmol) was added and the mixture heated to 70 °C and stirred at this temperature for 24 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (2.5-10% methanol in dichloromethane) to give **3c** as a colourless powder (51 mg, 28%) and **39c** as a colourless powder (8 mg, ~3%, impure).

6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (3c)



3c

^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.11 (s, 1H, NH), δ 8.00 (s, 1H, H-2), 6.39 (s, 2H, NH_2), 3.55 (d, *J* = 7.3 Hz, 2H, H-10), 1.80 (ddd, *J* = 10.7, 7.3, 3.4 Hz, 1H, H-11), 1.63 – 1.51 (m, 5H, H-12, H-13 and H-14), 1.32 – 0.74 (m, 5H, H-12, H-13 and H-14).

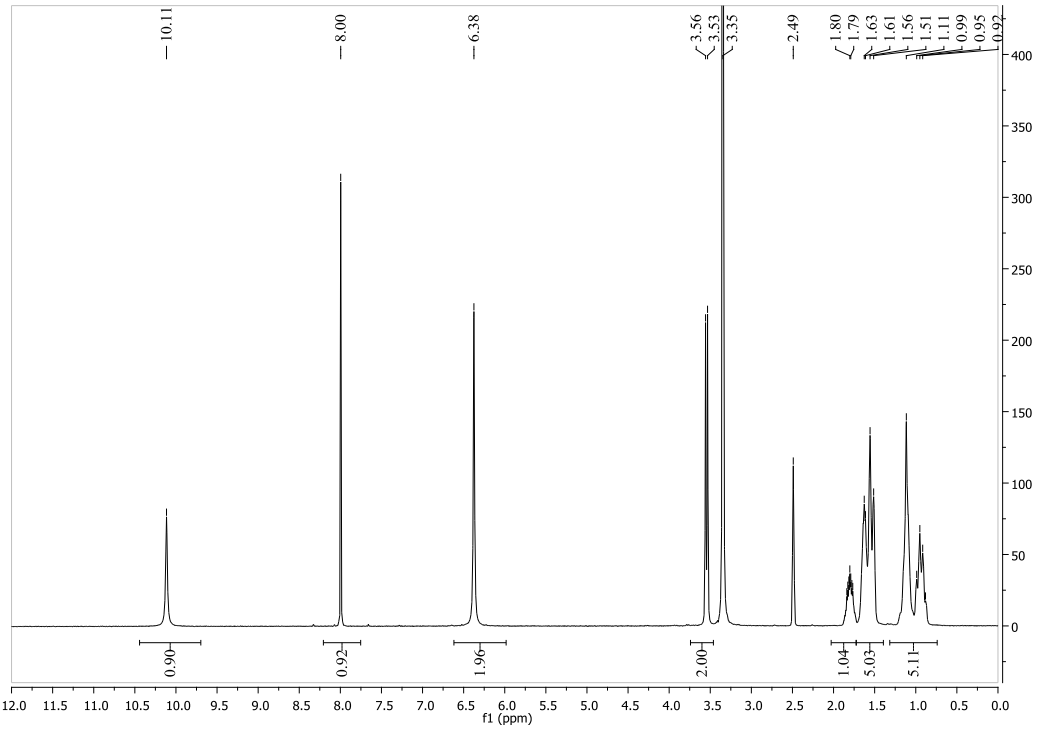
^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.3 (C-8), 150.9 (C-2), 147.8 (C-4), 146.5 (C-6), 103.1 (C-5), 45.1 (C-10), 36.3 (C-11), 30.1 (C-12), 25.9 (C-14), 25.1 (C-13).

MS EI *m/z* (rel. %) 247 (48, M^+), 231 (49), 165 (53), 151 (100), 136 (31).

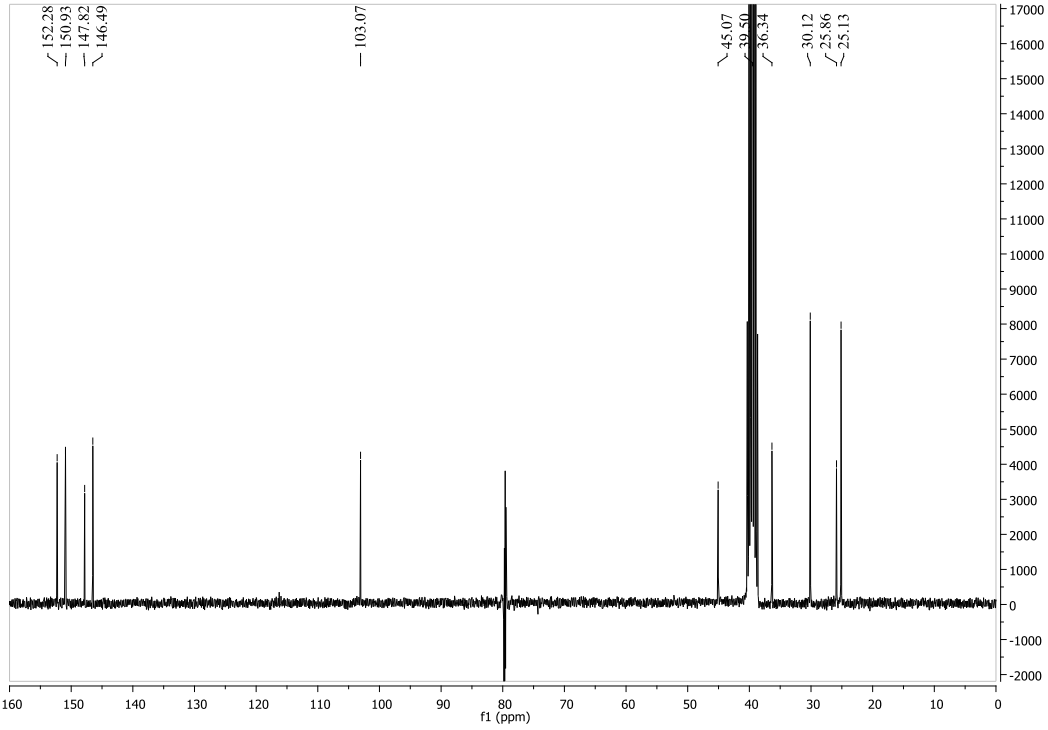
HR-MS Found 247.1422, calculated for $C_{12}H_{17}N_5O$ 247.1433.

Elem. Anal. Found C, 58.14; H, 6.83; N, 28.27. $C_{12}H_{17}N_5O$ requires C, 58.28; H, 6.93; N, 28.32.

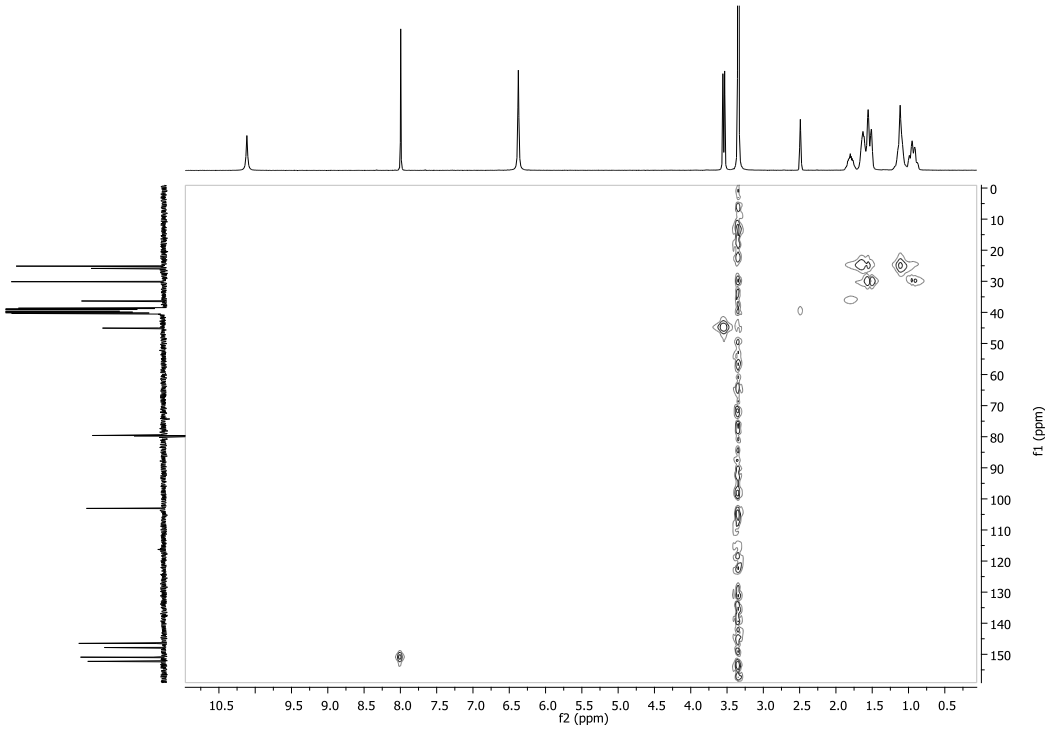
M.p. 287-289 °C.



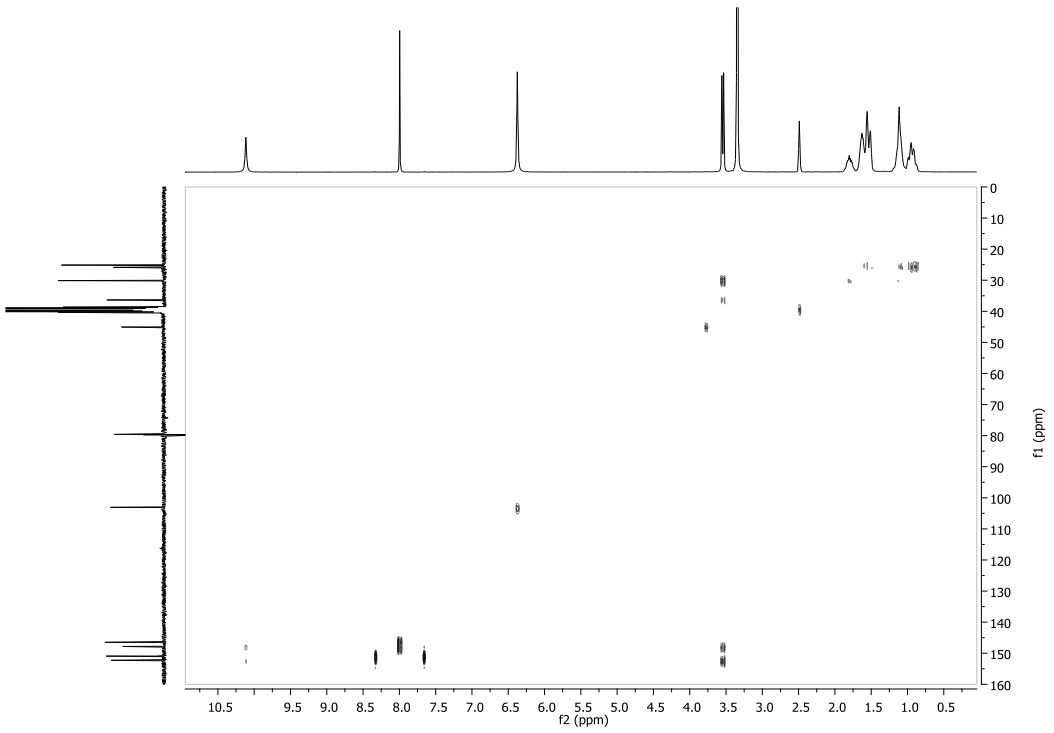
Spectrum 137. [1]H NMR of 6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**3c**).



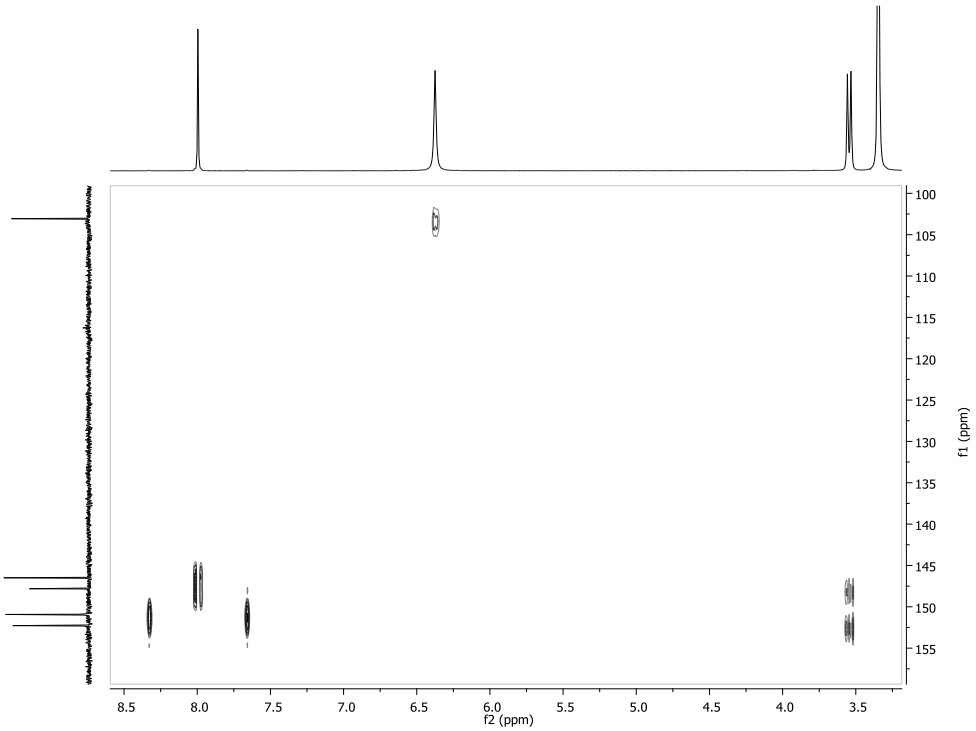
Spectrum 138. [13]C NMR of 6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**3c**).



Spectrum 139. HMQC of 6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**3c**).

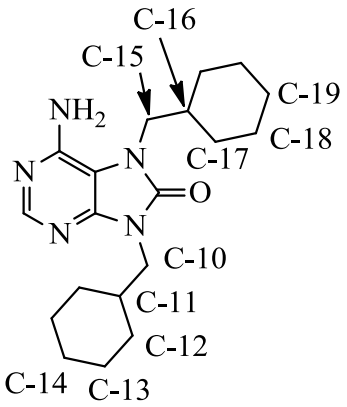


Spectrum 140. HMBC of 6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**3c**).



Spectrum 141. HMBC of 6-Amino-9-(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**3c**), expansion of the aromatic region.

6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (39c)



39c

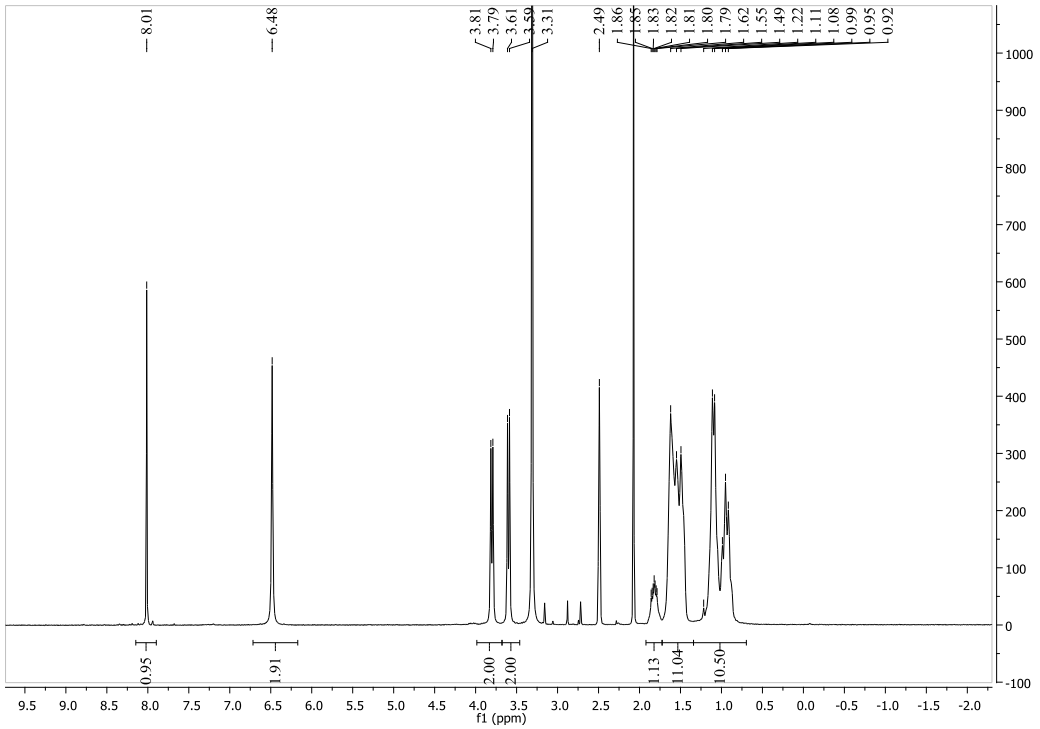
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.01 (s, 1H, H-2), 6.48 (s, 2H, NH_2), 3.80 (d, J = 7.4 Hz, 2H, C-10), 3.60 (d, J = 7.3 Hz, 2H, C-15), 1.92 – 1.72 (m, 2H), 1.72 – 1.34 (m, 22H), 1.04 (dt, J = 21.7, 21.3 Hz, 21H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.8 (C-8), 150.7 (C-2), 147.5 (C-4), 146.8 (C-6), 104.3 (C-5), 46.5 (C-15), 45.4 (C-10), 36.2, 30.7, 30.0, 29.4, 25.8, 25.14 and 25.05 (C-12 to C-14 and C-17 to C-19).

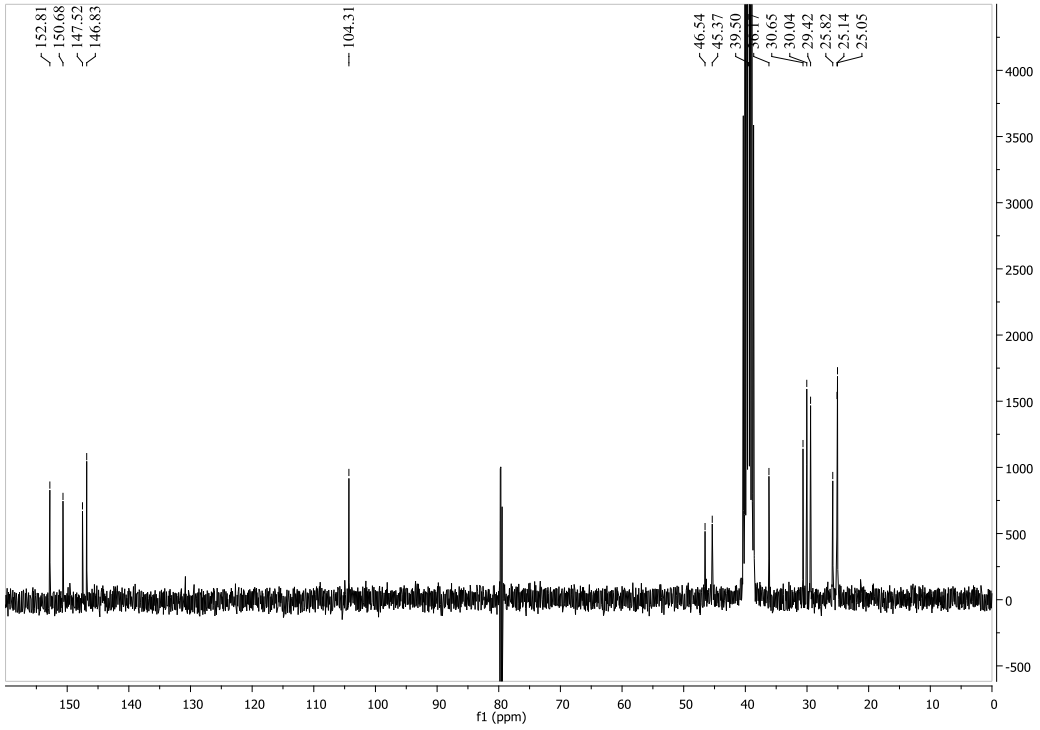
MS EI *m/z* (rel. %) 343 (100, M^+), 261 (56), 247 (49), 232 (10), 230 (8), 164 (42), 151 (49), 136 (7).

HR-MS Found 343.2362, calculated for $C_{19}H_{29}N_5O$ 343.2372.

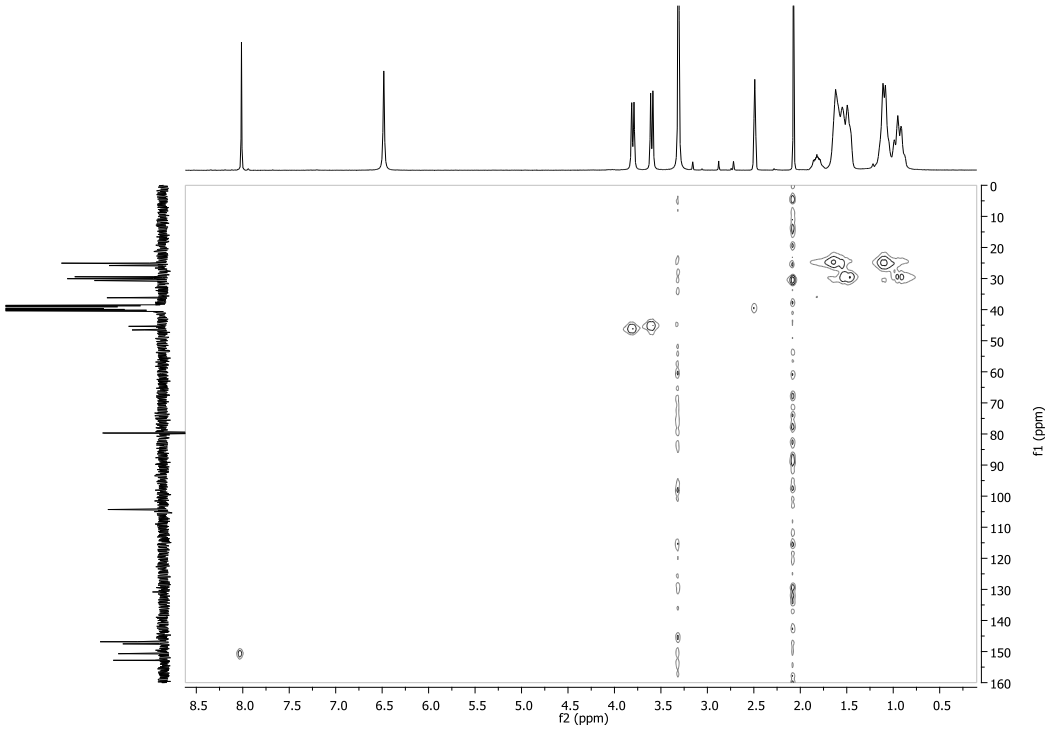
M.p. not obtained.



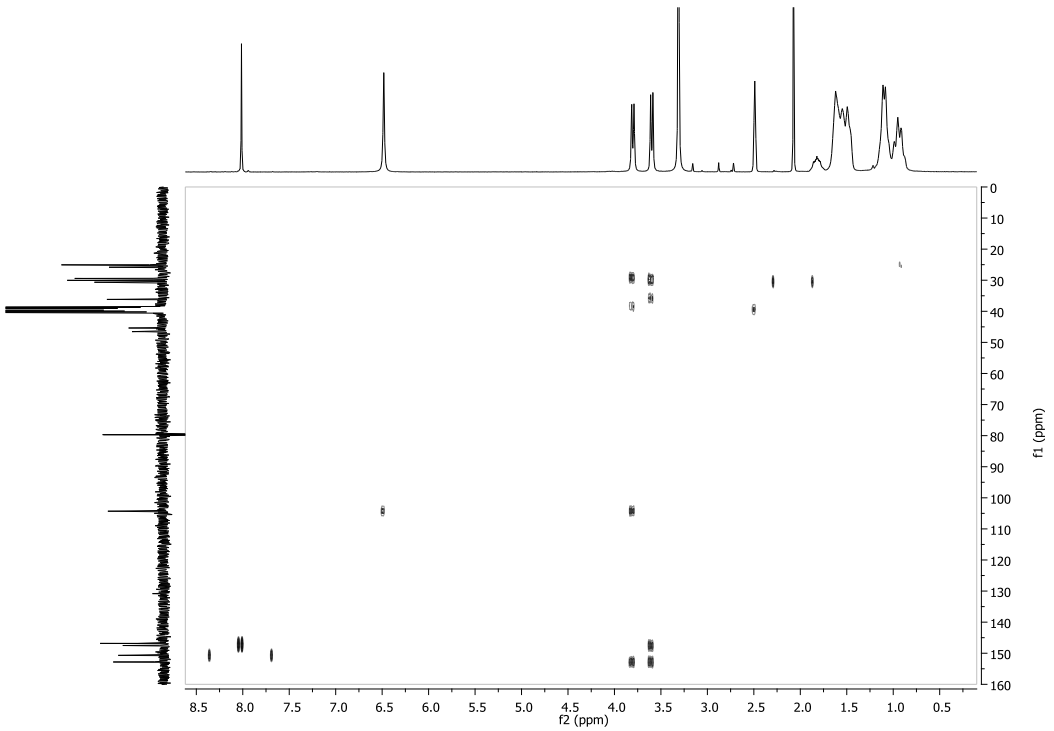
Spectrum 142. [1]H NMR of 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**39c**).



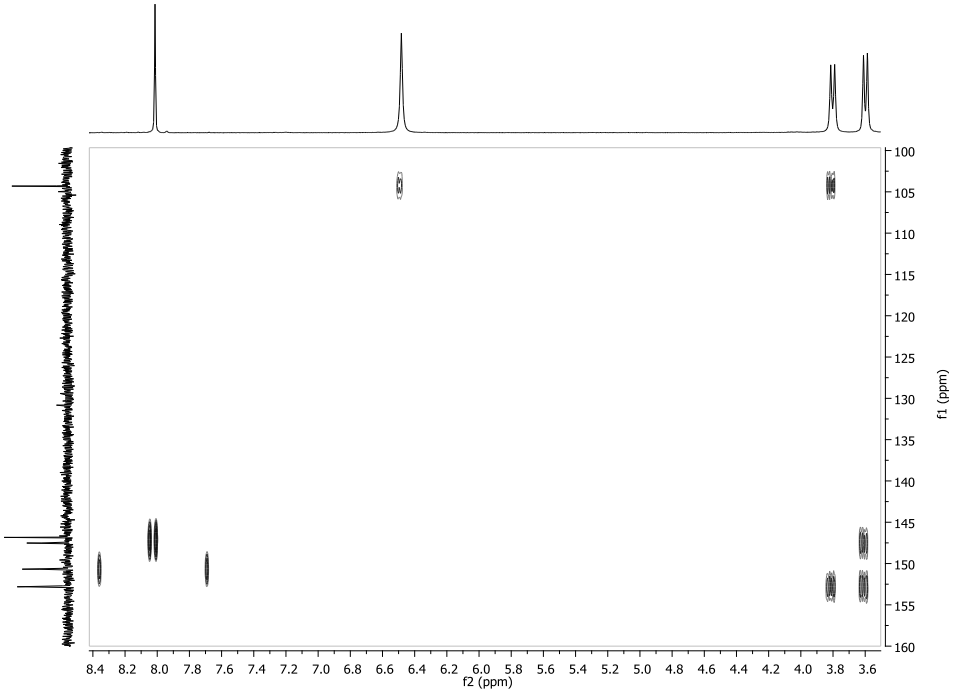
Spectrum 143. [13]C NMR of 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**39c**).



Spectrum 144. HMQC of 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**39c**).



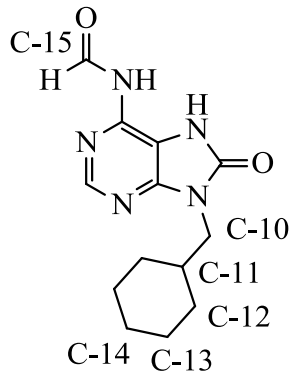
Spectrum 145. HMBC of 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**39c**).



Spectrum 146. HMBC of 6-Amino-7,9-bis(cyclohexylmethyl)-*7H*-purin-8(*9H*)-one (**39c**), expansion of the aromatic region.

Unknown by-product from hydrolysis of 9-(cyclohexylmethyl)-8-chloroadenine (48c)

Suggested structure:



48c

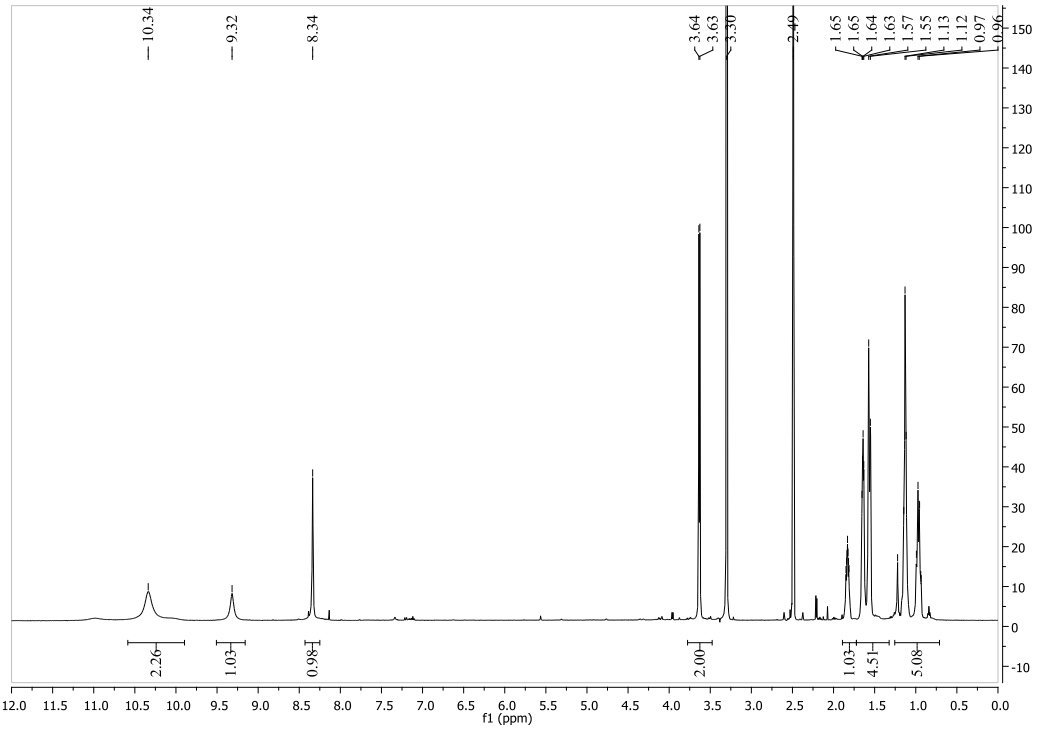
^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.99 (br s, 1H, NH or OH), 10.34 (br s, 2H, NH or OH), 9.32 (br s, 1H, NH or OH), 8.34 (s, 1H), 3.63 (d, J = 7.3 Hz, 2H), 1.83 (ddd, J = 11.0, 7.4, 3.6 Hz, 1H, H-11), 1.72 – 1.49 (m, 5H, H-12, H-13 and H-14), 1.16 – 0.90 (m, 5H, H-12, H-13 and H-14).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 161.8 (C-15), 152.3 (C-8), 150.5 (C-4), 150.2 (C-2), 137.7 (C-6), 106.3 (C-5), 45.3 (C-10), 36.2 (C-11), 30.0 (C-12), 25.8 (C-13), 25.1 (C-14).

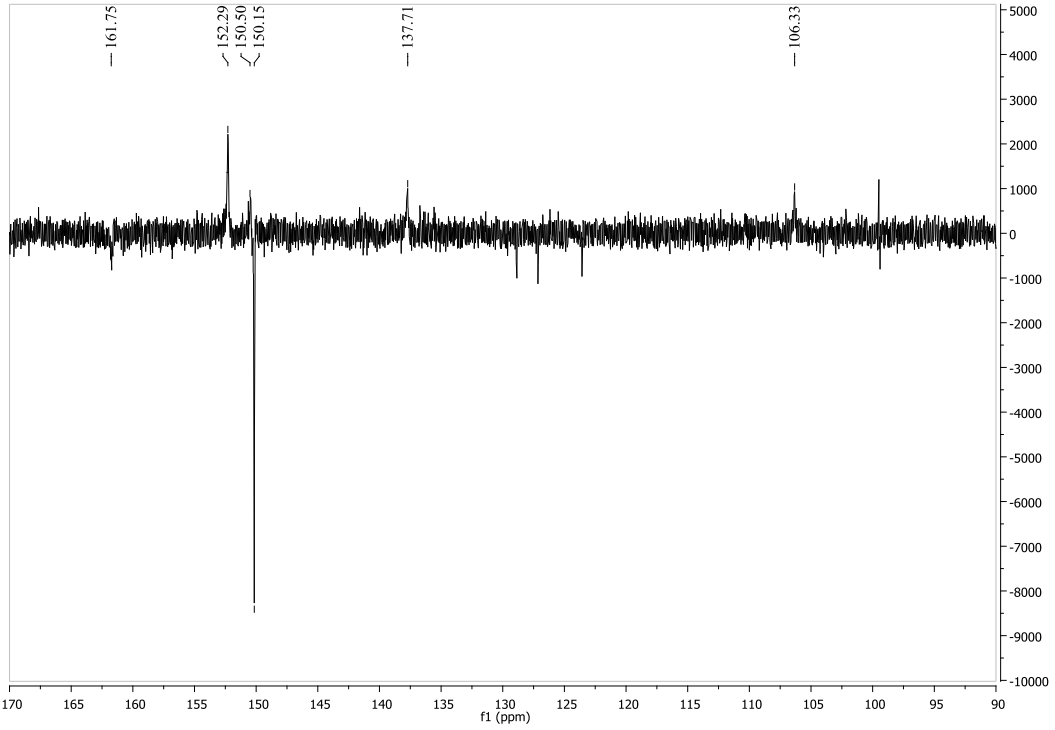
MS EI *m/z* (rel. %) 275 (27, M^+), 231 (7), 193 (30), 180 (100), 165 (14), 164 (16), 152 (21), 151 (20), 136 (7).

HR-MS Found 275.1376, calculated for $C_{13}H_{17}N_5O_2$ 275.1382.

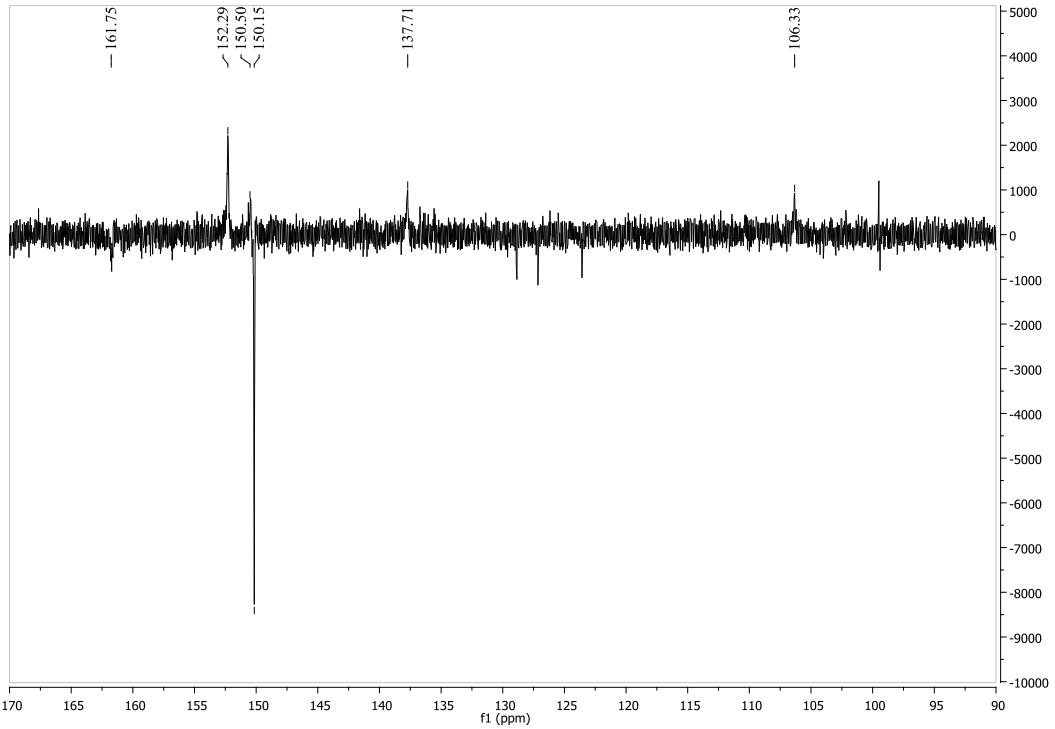
M.p. not obtained.



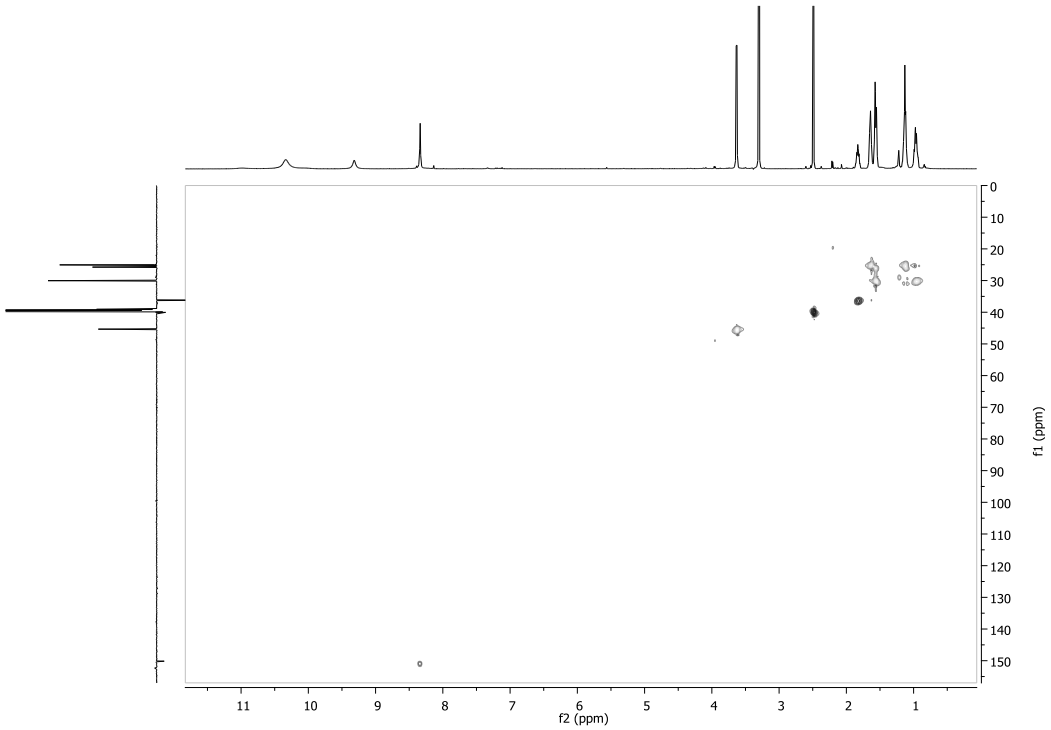
Spectrum 147. ^{1}H NMR of Unknown By-product (**48c**).



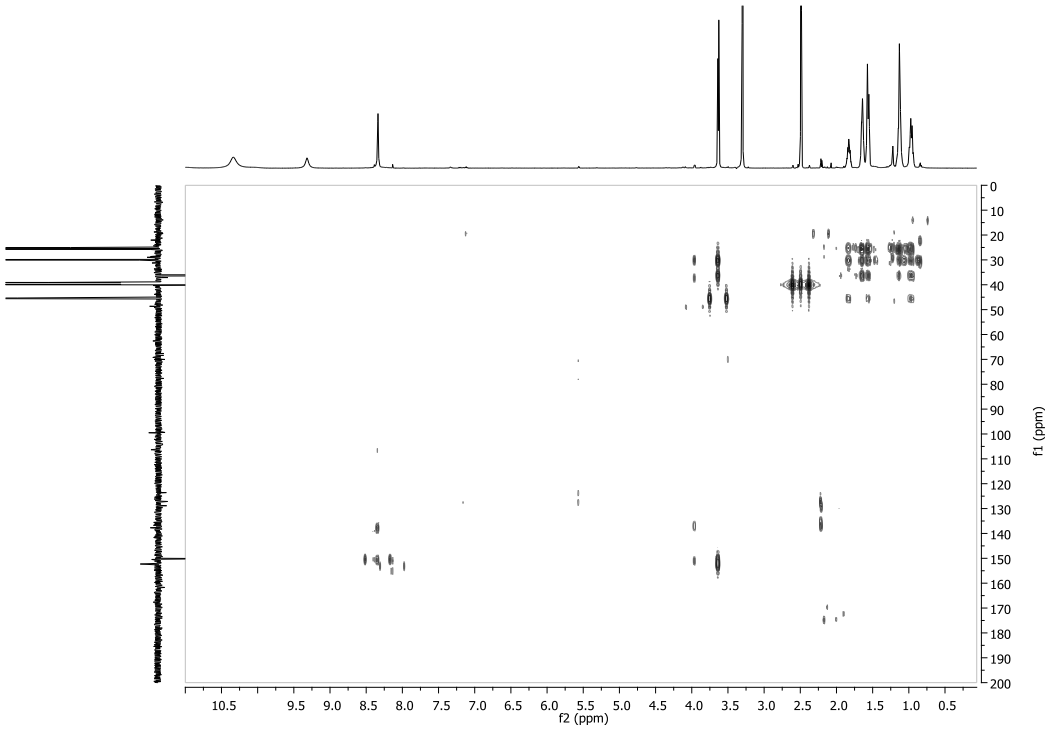
Spectrum 148. ^{13}C APT NMR of Unknown By-product (**48c**).



Spectrum 149. ^{13}C APT NMR of Unknown By-product (**48c**). Signals which give correlations and/or appear to be "real" signals are indicated.

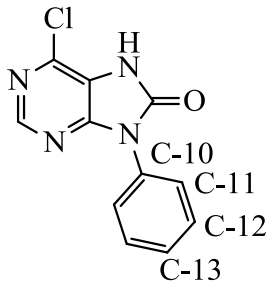


Spectrum 150. HSQC of Unknown By-product (**48c**).



Spectrum 151. HMBC of Unknown By-product (**48c**).

6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (11)



11

6-Chloro-N^4-phenylpyrimidine-4,5-diamine (**10a**) (440 mg, 2.00 mmol) and carbon diimidazole (520 mg, 3.21 mmol) was refluxed for 6 h in dry THF (5 mL). The solvent was evaporated *in vacuo* and the residue purified using flash chromatography (0-1% methanol in dichloromethane) to give **11** as a colourless powder (480 mg, 97%).

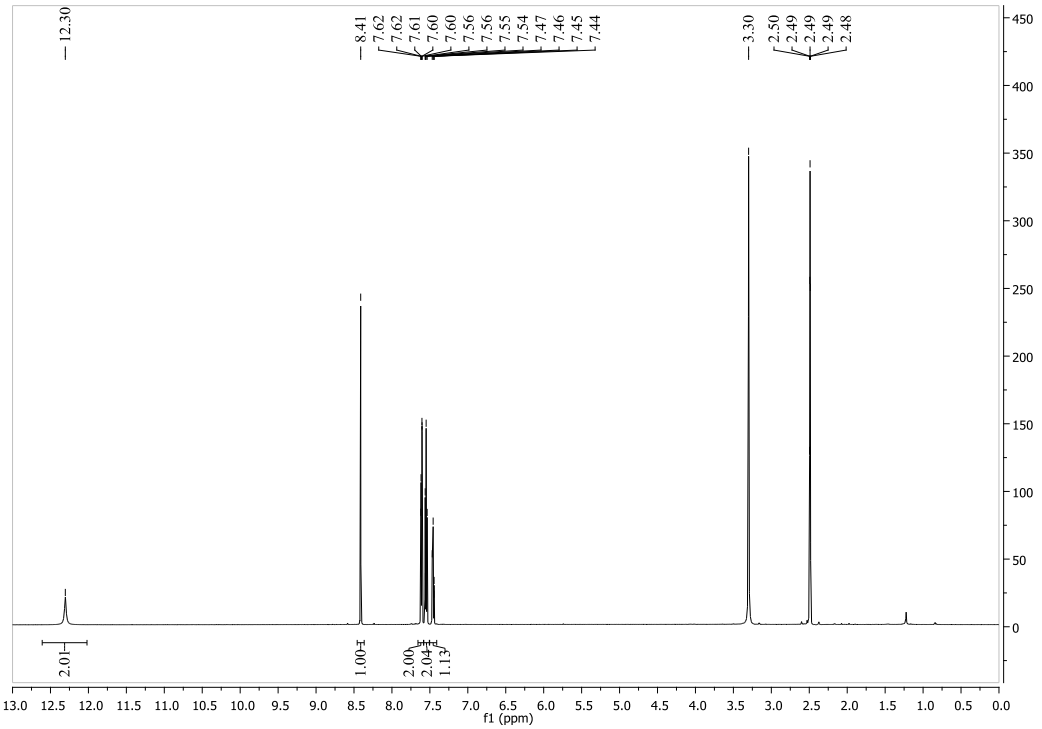
^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.30 (br s, 1H, NH), 8.41 (s, 1H, H-2), 7.66 – 7.58 (m, 2H, H-11), 7.56 – 7.54 (m, 2H, H-12), 7.47 – 7.44 (m, 1H, H-13).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 152.0 (C-8), 150.6 (C-4 or C-6), 150.1 (C-2), 134.9 (C-4 or C-6), 132.4 (C-10), 128.9 (C-12), 128.1 (C-13), 126.3 (C-11), 119.9 (C-5).

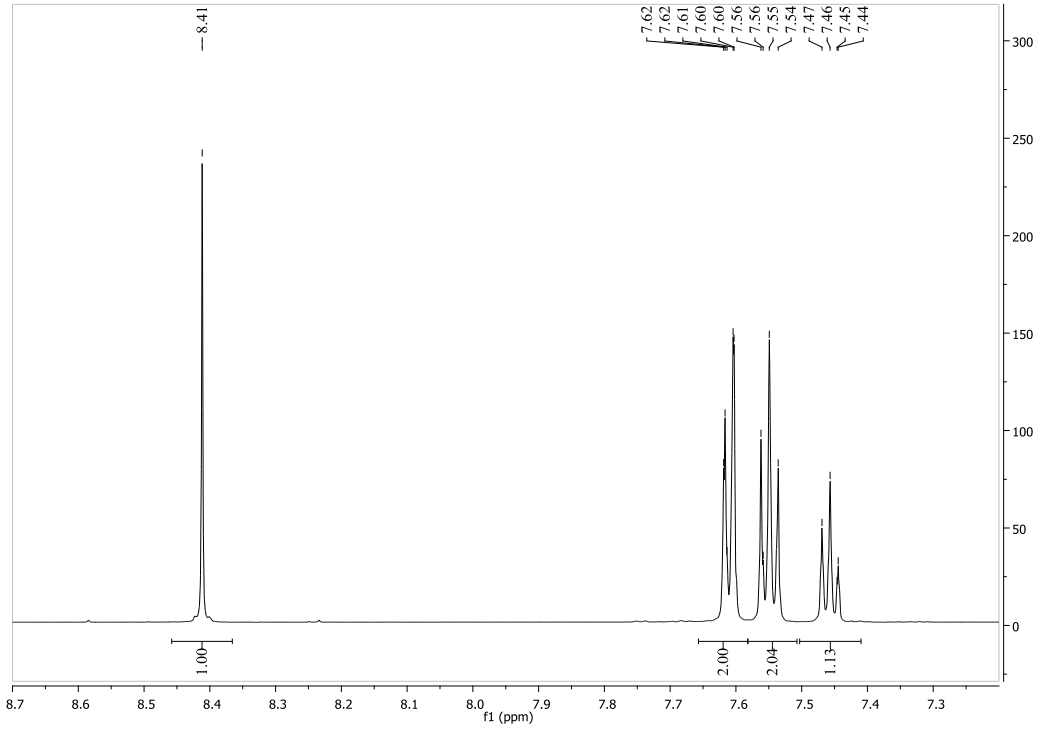
MS EI *m/z* (rel. %) 248/246 (32/100, M^+), 247/245 (50/96), 103 (7), 77 (17), 51 (7).

HR-MS Found 246.0302, calculated for $C_{11}H_7ClN_4O$ 246.0308.

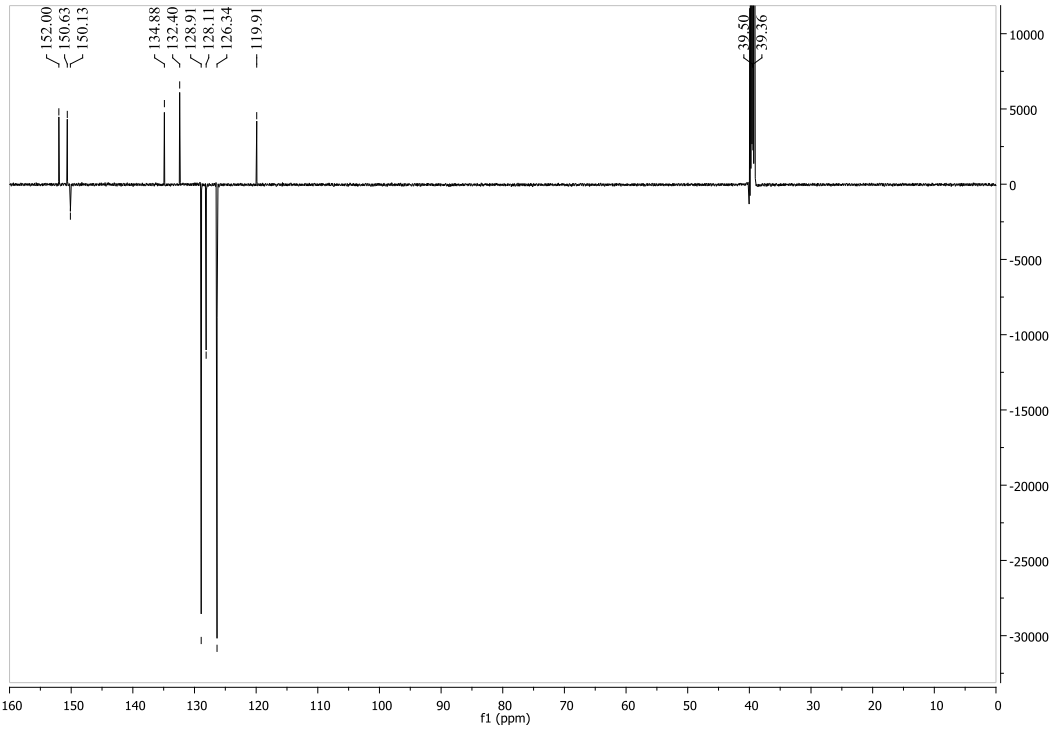
M.p. 293 °C.



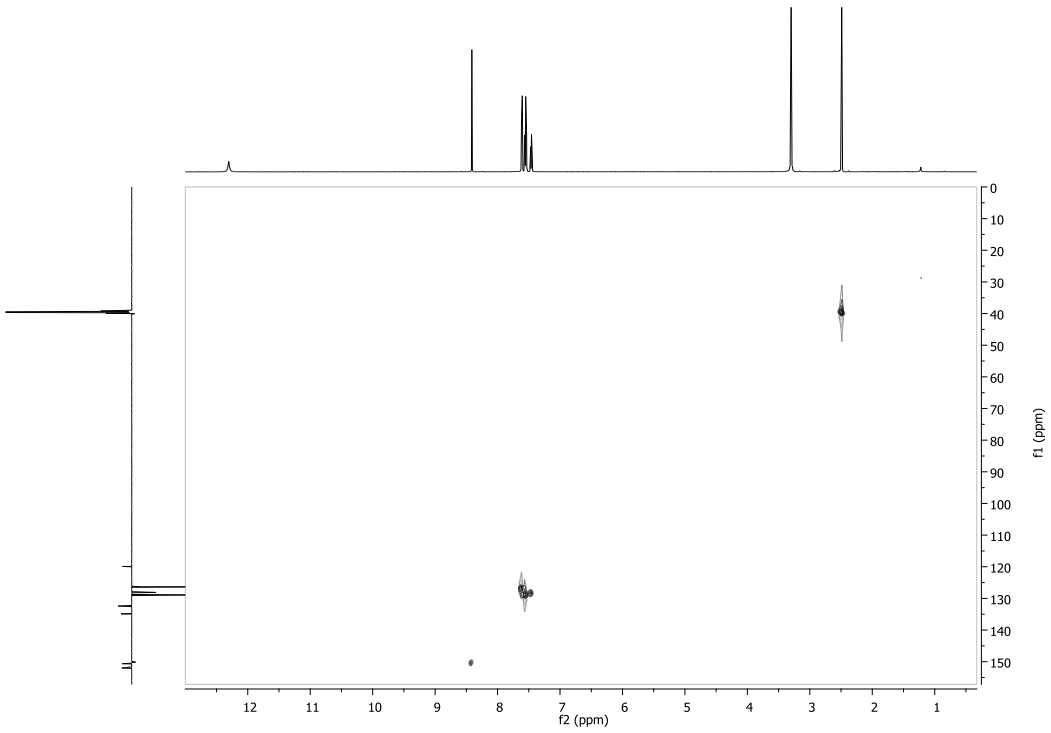
Spectrum 152. [1]H NMR of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**).



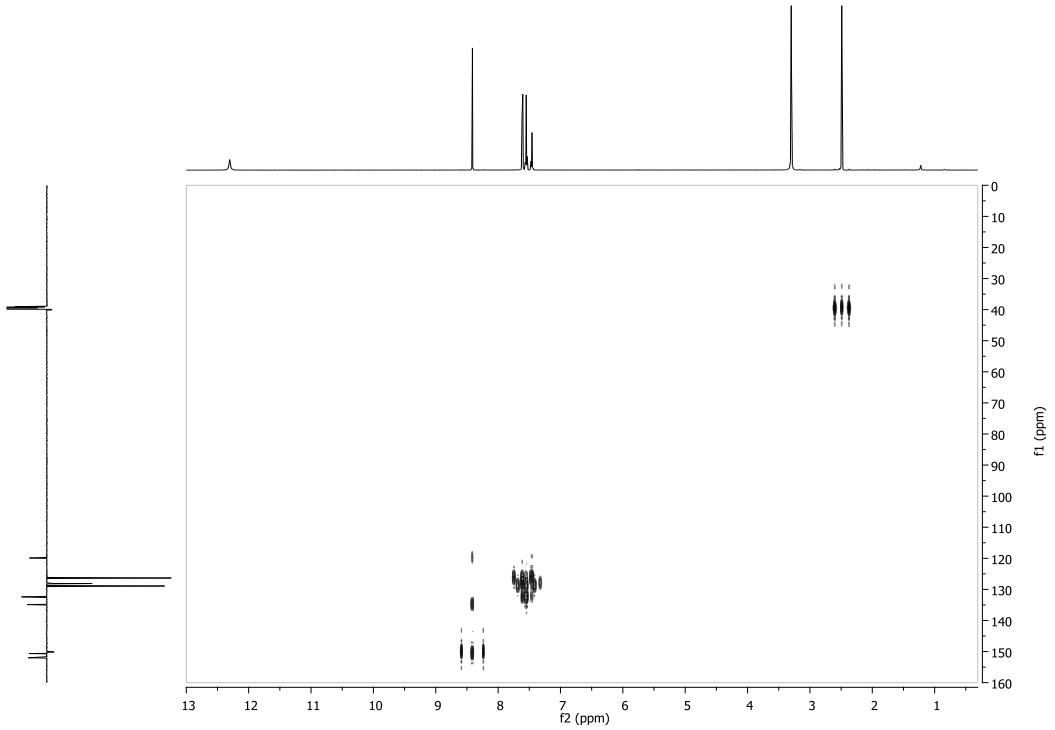
Spectrum 153. [1]H NMR of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**), expansion of the aromatic region.



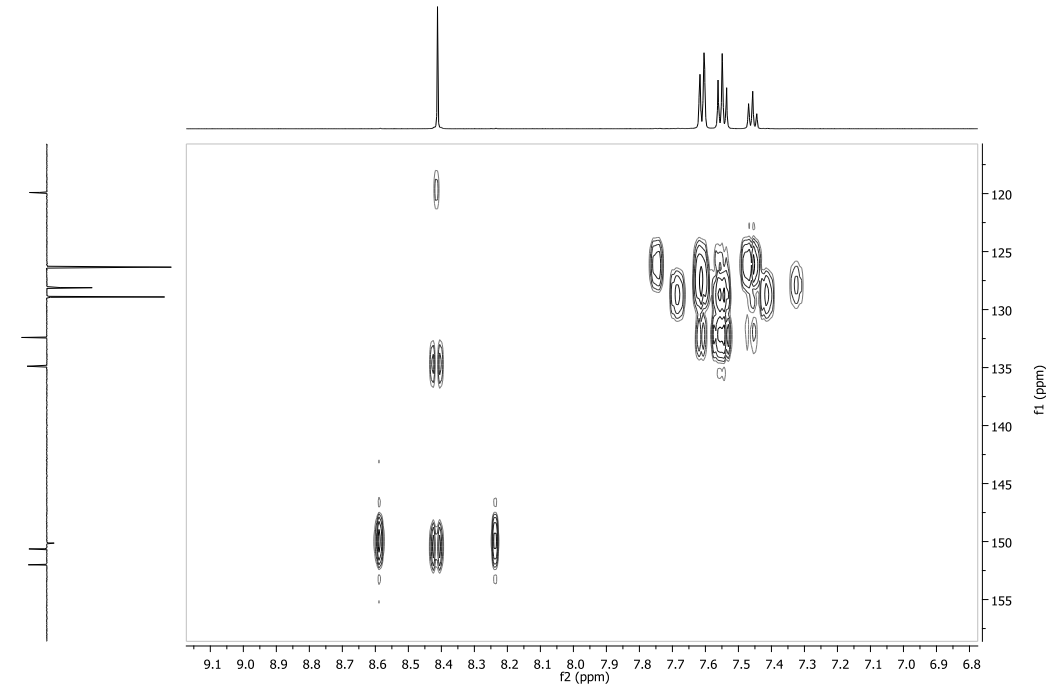
Spectrum 154. ^{13}C APT NMR of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**).



Spectrum 155. HSQC of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**).

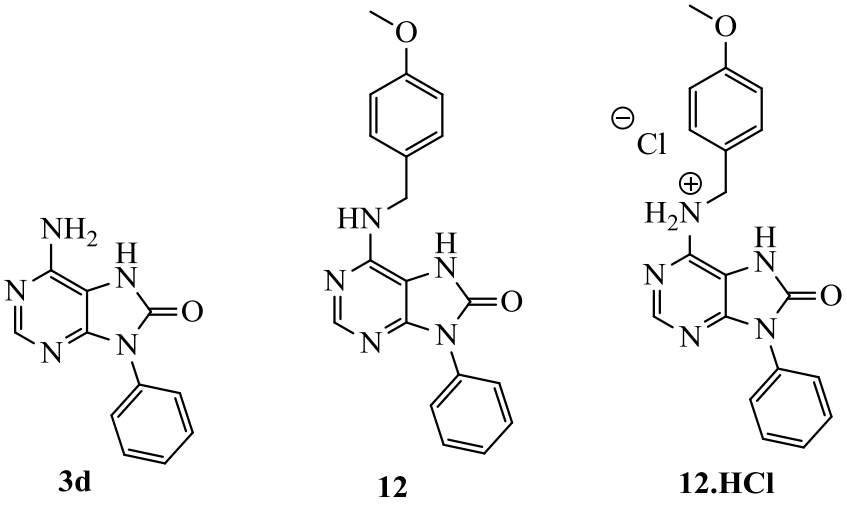


Spectrum 156. HMBC of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**).



Spectrum 157. HMBC of 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**), expansion of the aromatic region.

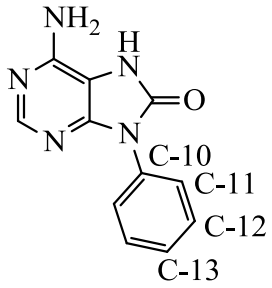
6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (3d), 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (12) and hydrochloride salt (12.HCl)



Method 1: 6-Chloro-9-phenyl-*7H*-purin-8(*9H*)-one (**11**) and para-methoxybenzylamine was refluxed in *n*-butanol for 24 h. The solvent was evaporated *in vacuo* and the residue was purified using flash chromatography (0-2% methanol in dichloromethane) to give **12** as a colourless power (345 mg, ~7% **12.HCl**). The crude product was then heated in trifluoroacetic acid (5 mL) at 60 °C for 2 h. The acid was evaporated *in vacuo* and the residue was purified using flash chromatography (0-10% methanol in dichloromethane) to give **3d** as a colourless powder (171 mg, 75% over two steps). It is possible to obtain pure **12** through recrystallization of the crude after the first step with chloroform, hexanes and ethanol with a yield of 52%. Its hydrochloride salt (**12.HCl**) can be isolated through flash chromatography in the second step.

Method 2: 8-Chloro-9-phenyladenine (**8d**) (146 mg, 0.594 mmol) was refluxed in formic acid (15 mL) overnight with stirring. The formic acid was evaporated then co-evaporated with 3 x 15 mL water and product dried *in vacuo*. The residue was purified using flash chromatography (0-10% methanol in dichloromethane) to give **3d** as a colourless powder (98 mg, 73%) and an unknown by-product (**48d**) as a colourless powder (6 mg, 4%).

6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (3d)



3d

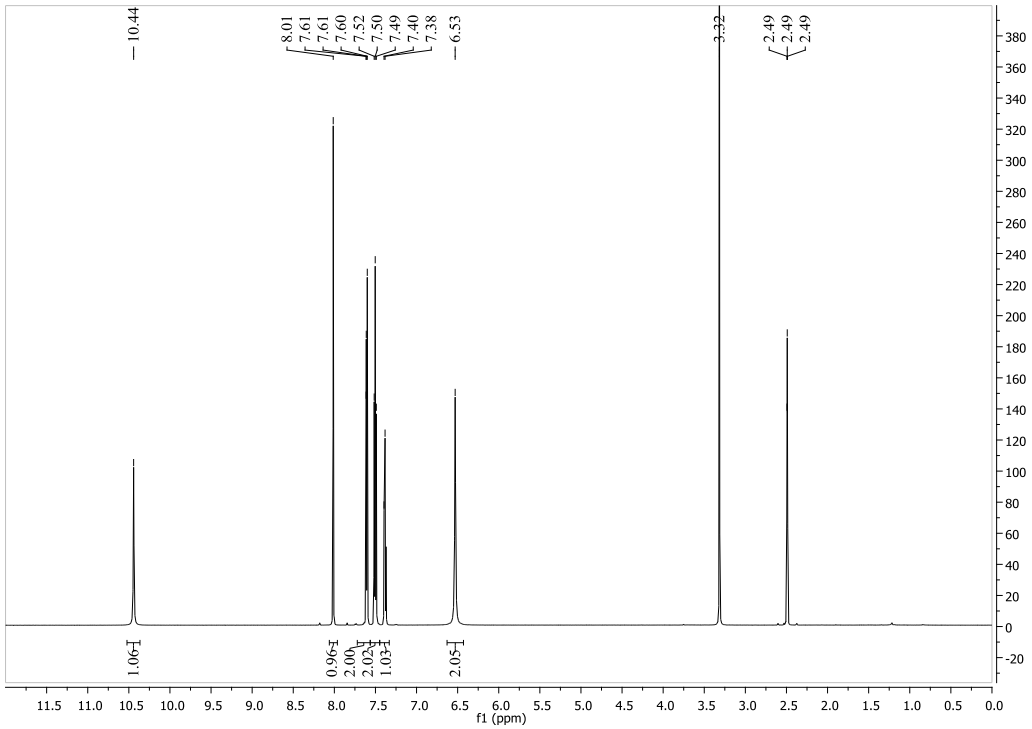
^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.44 (br s, 1H, NH), 8.01 (s, 1H, H-2), 7.72 – 7.56 (m, 2H, H-11), 7.52 – 7.49 (m, 2H, H-12), 7.40 – 7.38 (m, 1H, H-13), 6.53 (br s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 151.56 (C-8), 151.55 (C-2), 147.589 (C-4 or C-6), 147.582 (C-4 or C-6), 133.9 (C-10), 129.2 (C-12), 127.7 (C-13), 126.4 (C-11), 104.0 (C-5).

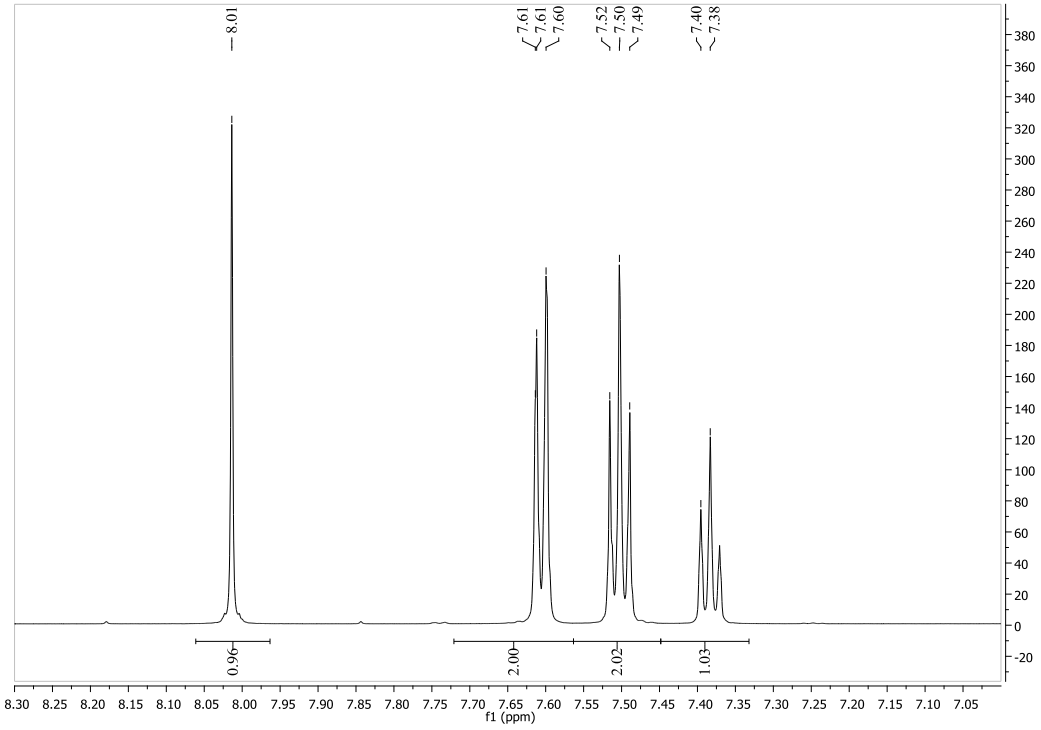
MS EI *m/z* (rel. %) 228 (23), 227 (100, M^+), 226 (42).

HR-MS Found 227.0802, calculated for $C_{11}H_9N_5O$ 227.0807.

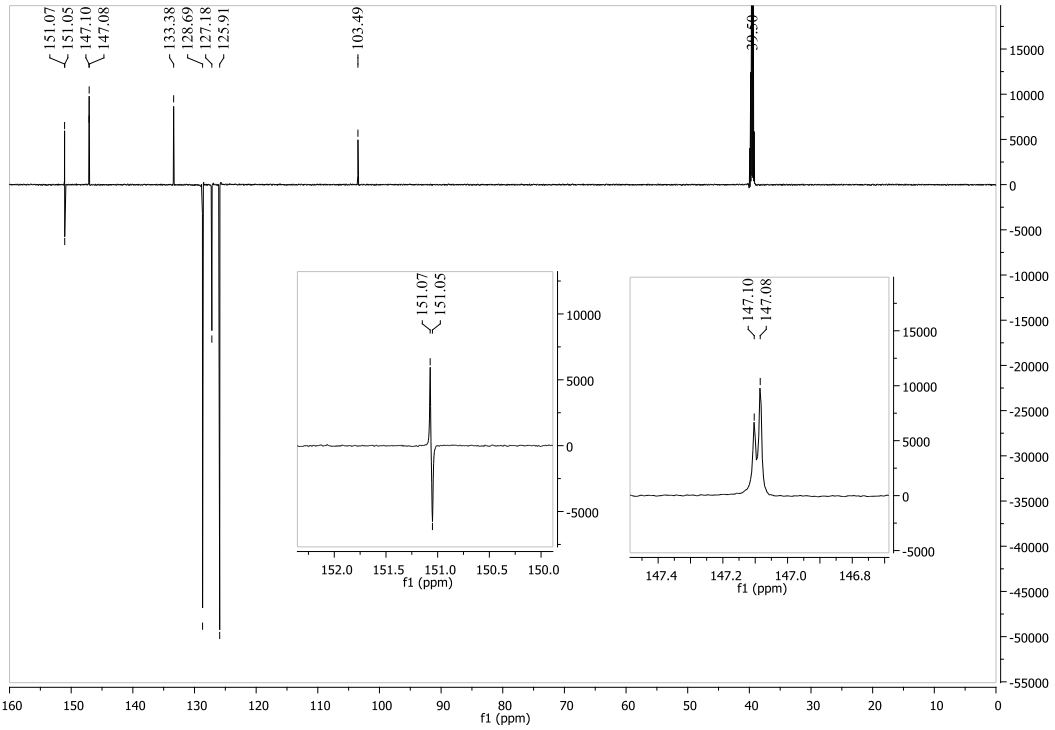
M.p. > 300 °C (decomposed) (lit.[99] > 300 °C, decomposed).



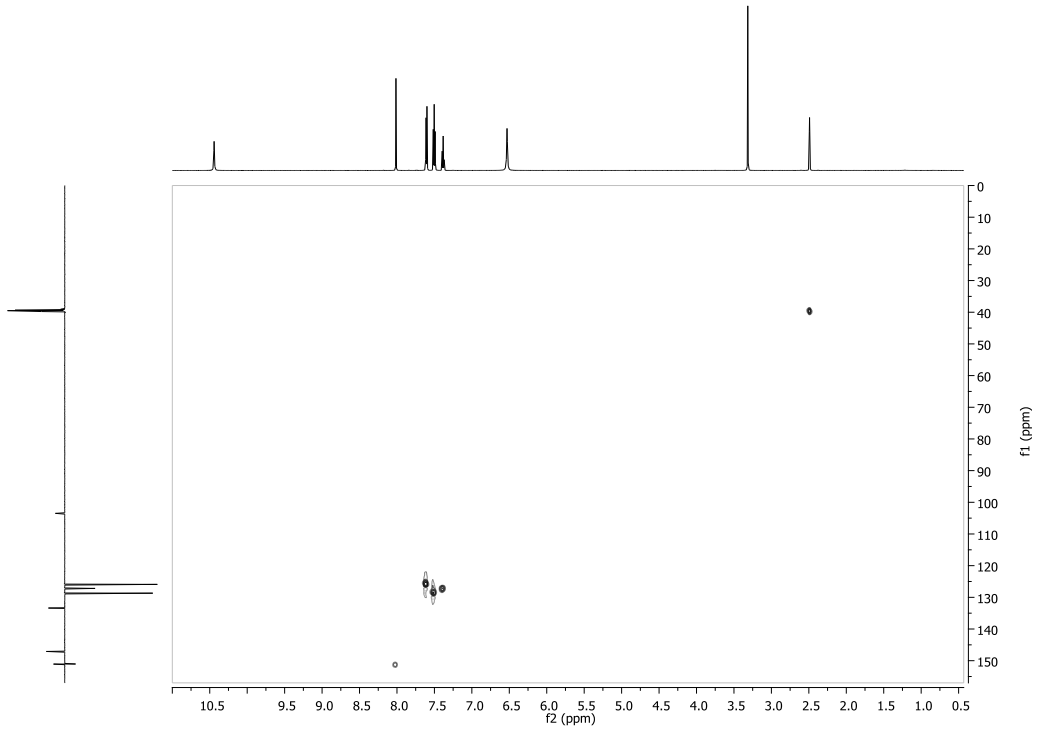
Spectrum 158. [1]H NMR of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**).



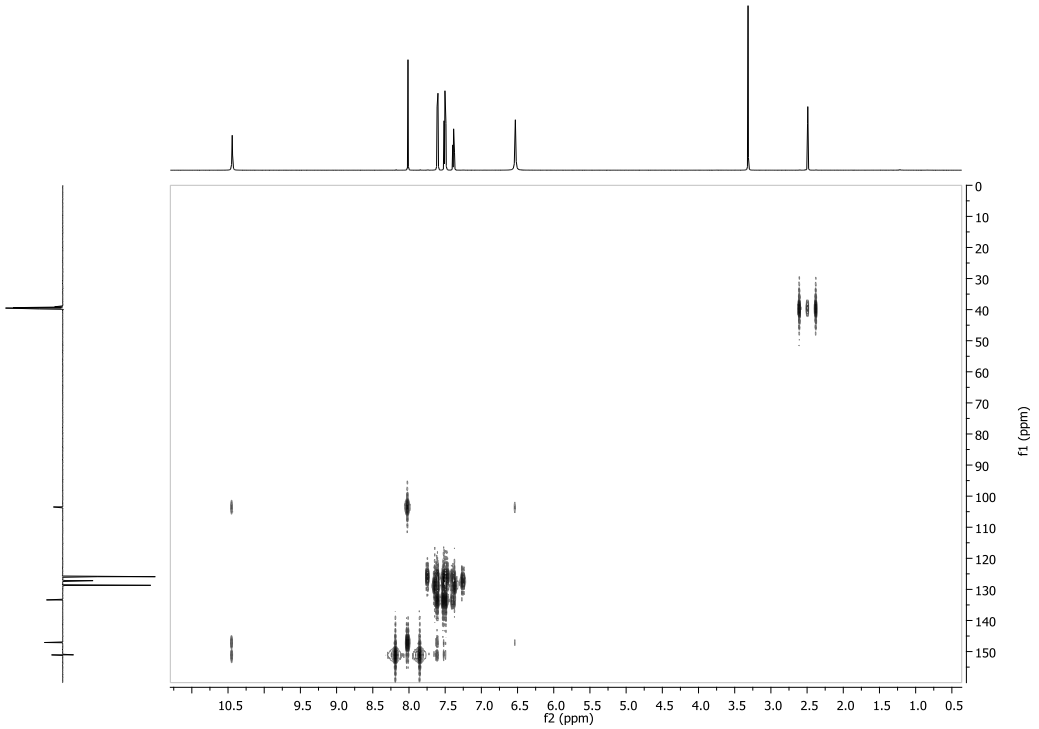
Spectrum 159. [1]H NMR of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**), expansion of the aromatic region.



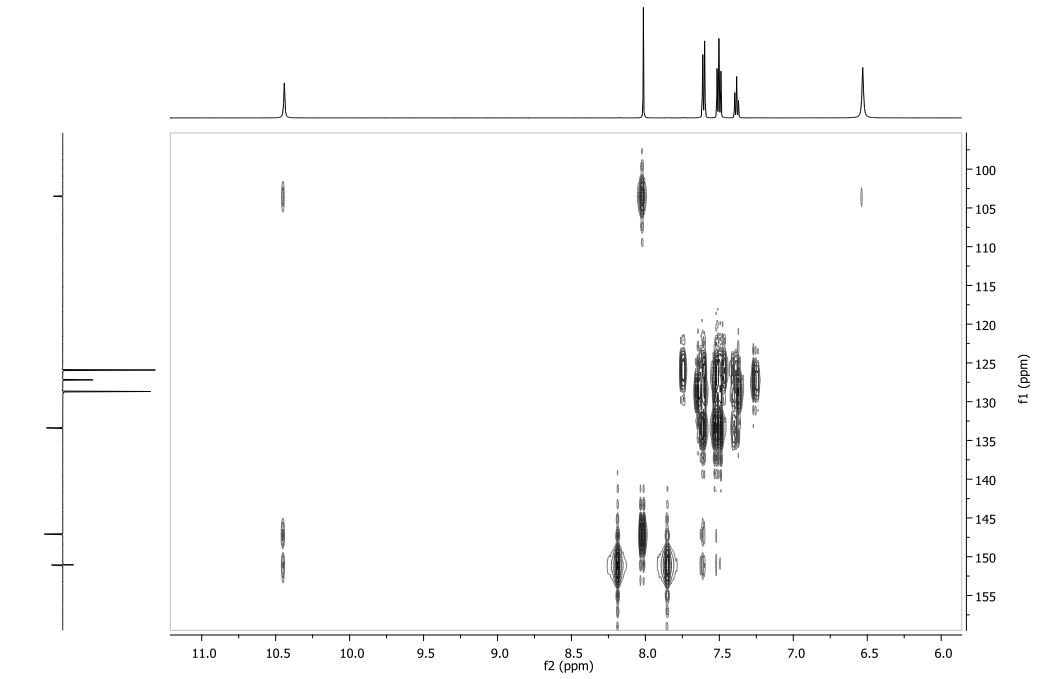
Spectrum 160. ^{13}C APT NMR of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**), with expansions clearly showing the four signals that are close-lying.



Spectrum 161. HSQC of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**).

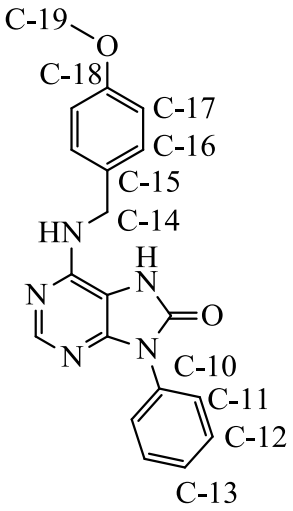


Spectrum 162. HMBC of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**).



Spectrum 163. HMBC of 6-Amino-9-phenyl-*7H*-purin-8(*9H*)-one (**3d**), expansion of the aromatic region.

6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (12)



12

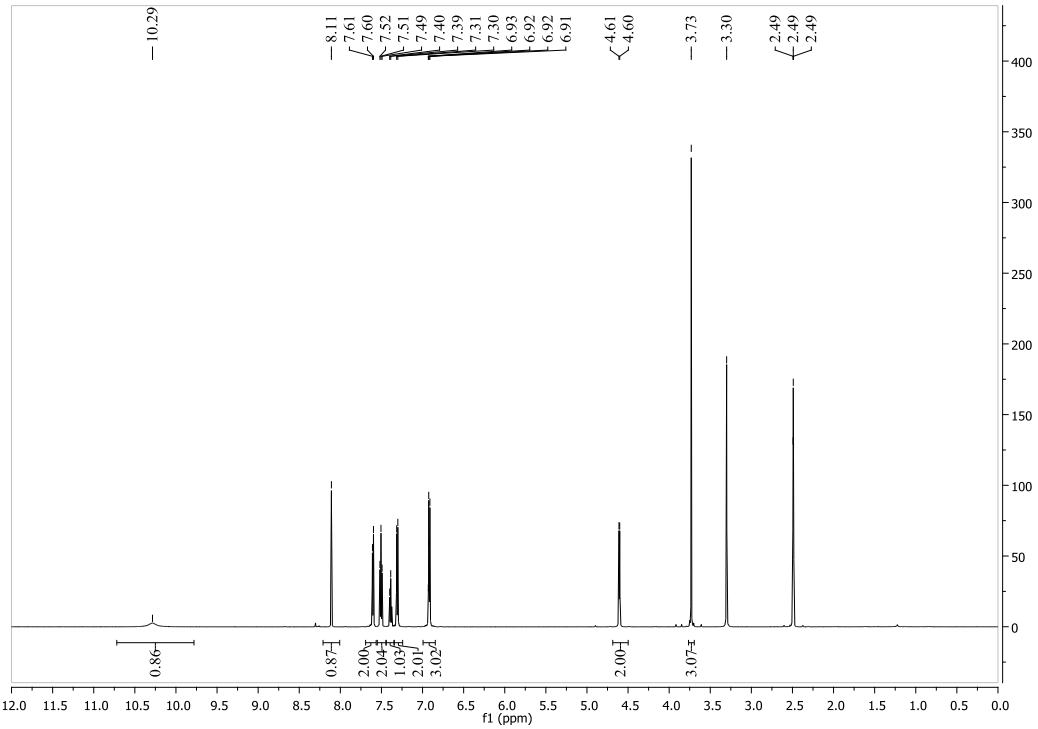
^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.29 (s, 1H, NH), 8.11 (s, 1H, H-2), 7.60 (d, *J* = 7.5 Hz, 2H, H-11), 7.51 (t, *J* = 7.9 Hz, 2H, H-12), 7.39 (d, *J* = 7.4 Hz, 1H, H-13), 7.31 (d, *J* = 8.6 Hz, 2H, C-16), 6.92 (dd + br s, *J* = 6.8, 4.8 Hz, 3H, H-17 and NH), 4.61 (d, *J* = 5.6 Hz, 2H, H-14), 3.73 (s, 3H, H-19).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 158.5 (C-19), 151.96 (C-2), 150.94 (C-8), 146.5 (C-4), 146.0 (C-6), 133.3 (C-10), 131.1 (C-15), 128.9 (C-16), 128.7 (C-12), 127.2 (C-13), 125.9 (C-11), 113.9 (C-17), 103.7 (C-5), 55.1 (C-19), 43.1 (C-14).

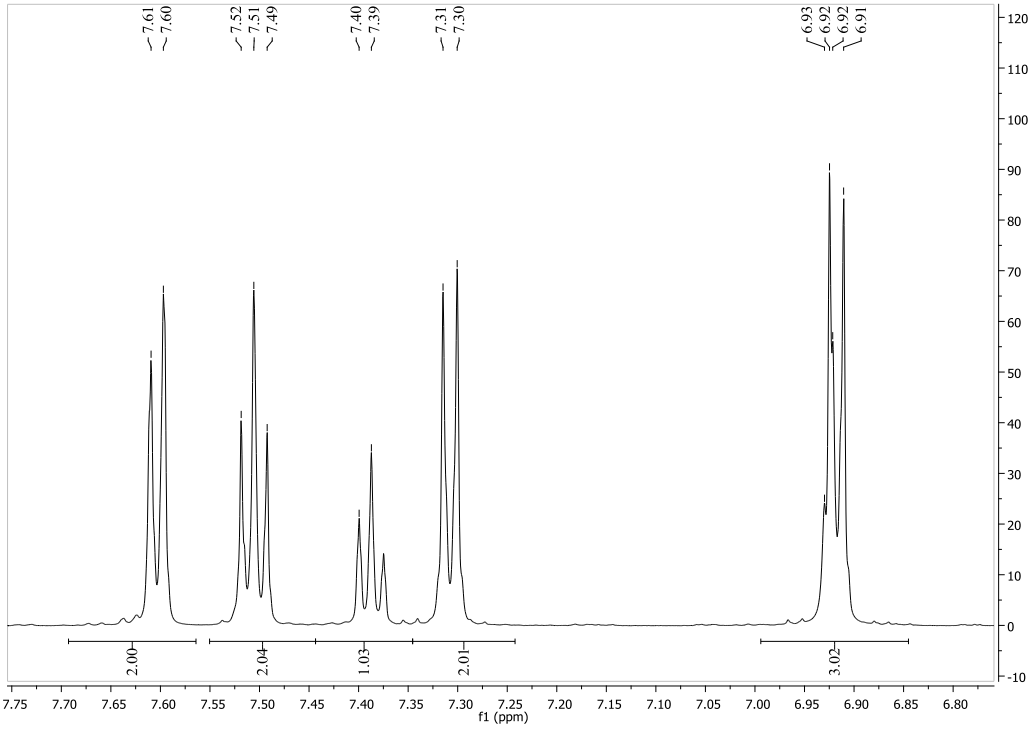
MS EI *m/z* (rel. %) 347 (45, M^+), 227 (9), 121 (100).

HR-MS Found 347.1382, calculated for $C_{19}H_{17}N_5O_2$ 347.1390.

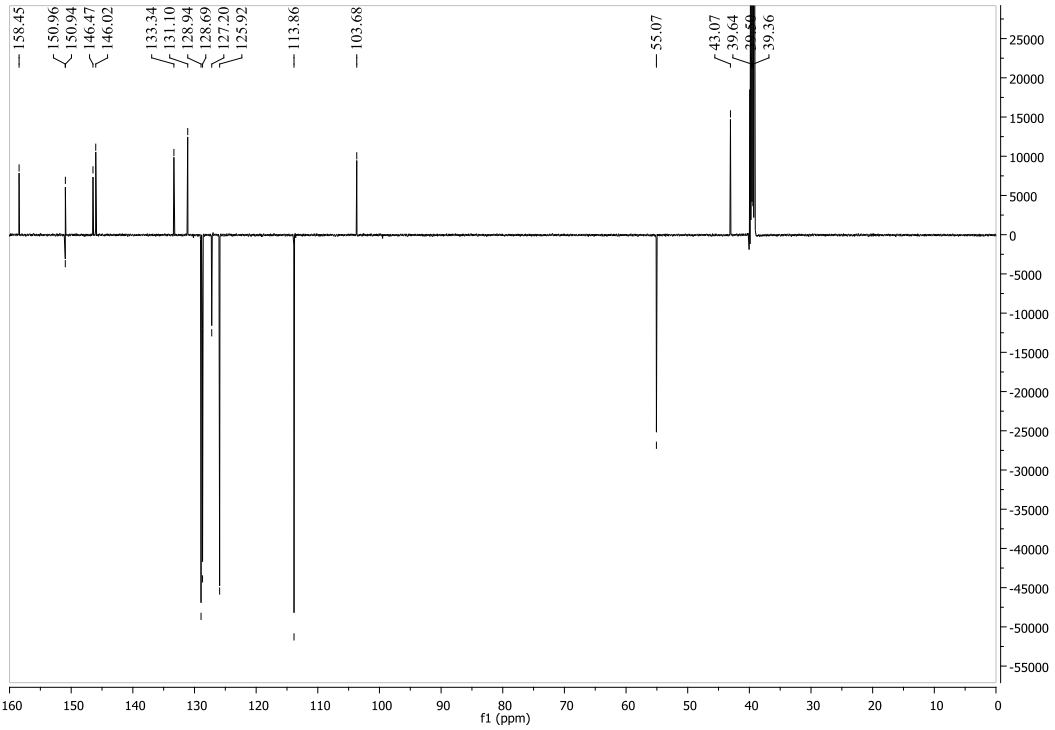
M.p. 223-227 °C



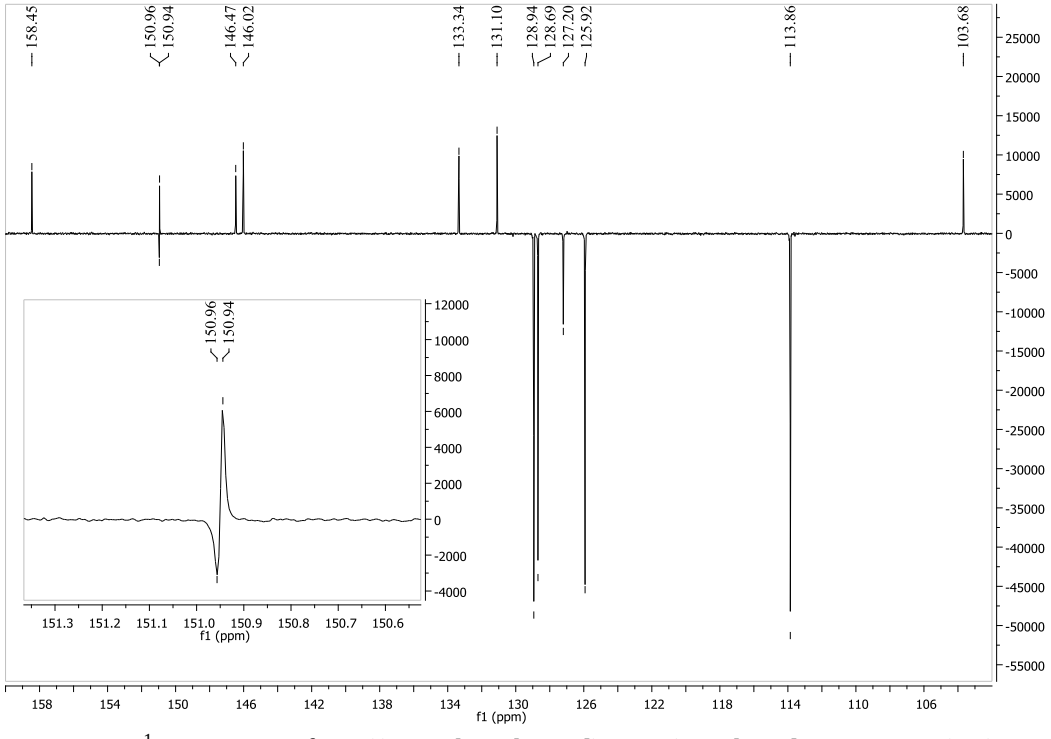
Spectrum 164. [1]H NMR of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**).



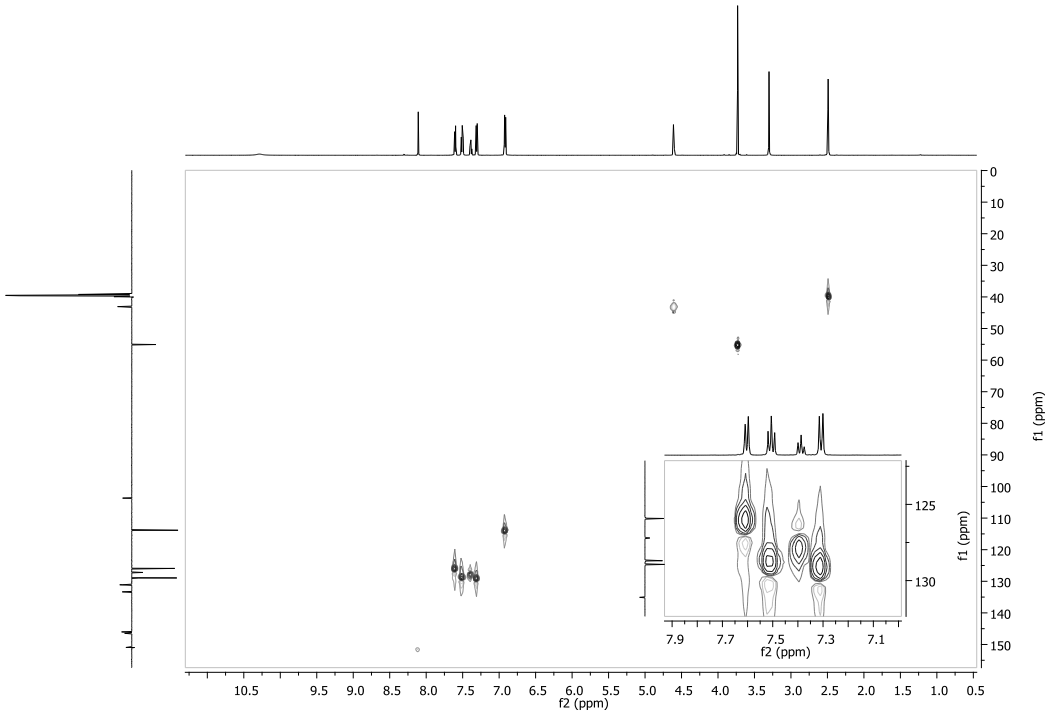
Spectrum 165. [1]H NMR of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**), expansion of the phenyl region.



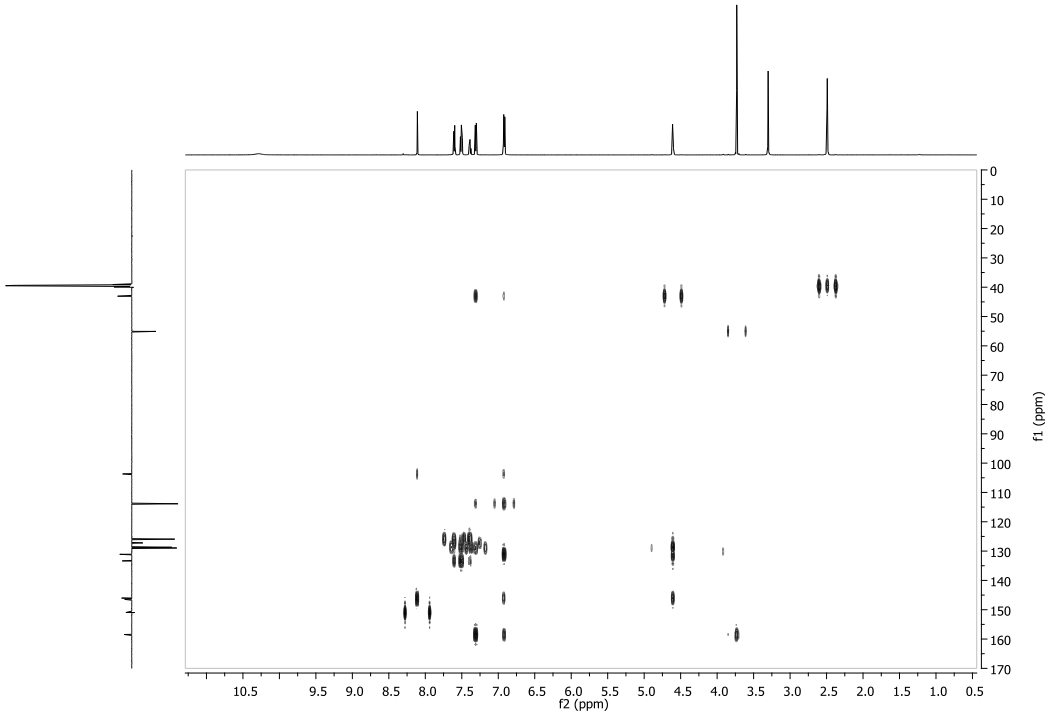
Spectrum 166. ^{13}C NMR of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**).



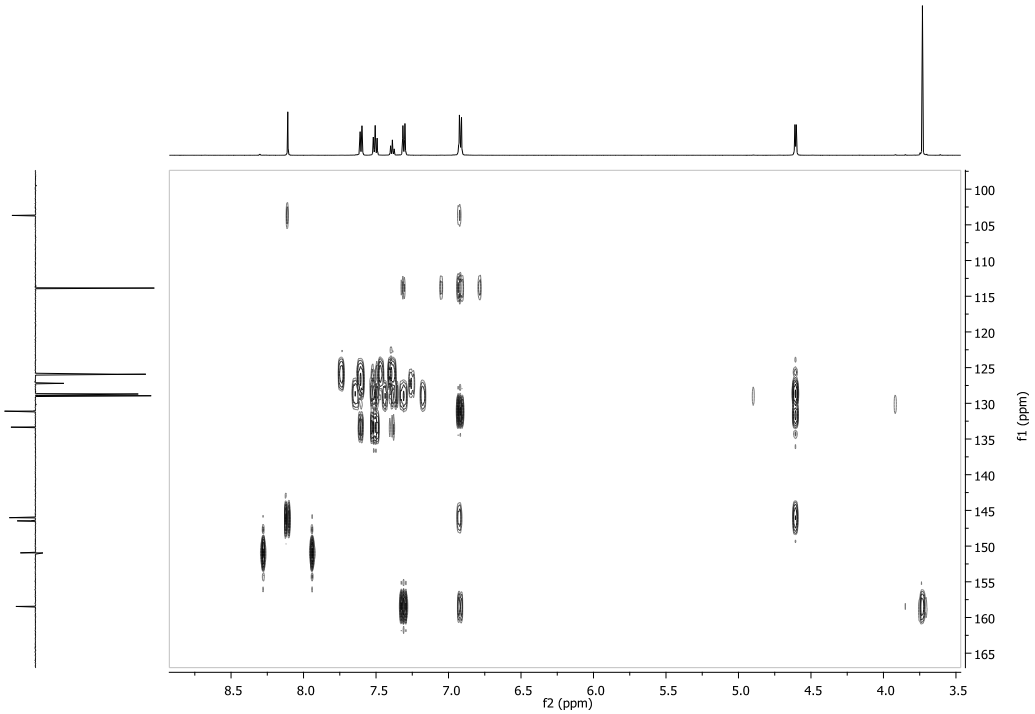
Spectrum 167. ^{1}H NMR of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**), expansion of the aromatic region with further expansion around 150.9 ppm.



Spectrum 168. HSQC of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**), with expansion of part of the phenyl region (inset).



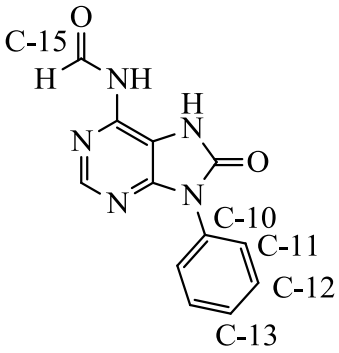
Spectrum 169. HMBC of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**).



Spectrum 170. HMBC of 6-((4-Methoxybenzyl)amino)-9-phenyl-*7H*-purin-8(*9H*)-one (**12**), expansion of the aromatic region.

Unknown By-product from hydrolysis of 8-chloro-9-phenyladenine (48d)

Suggested structure:



48d

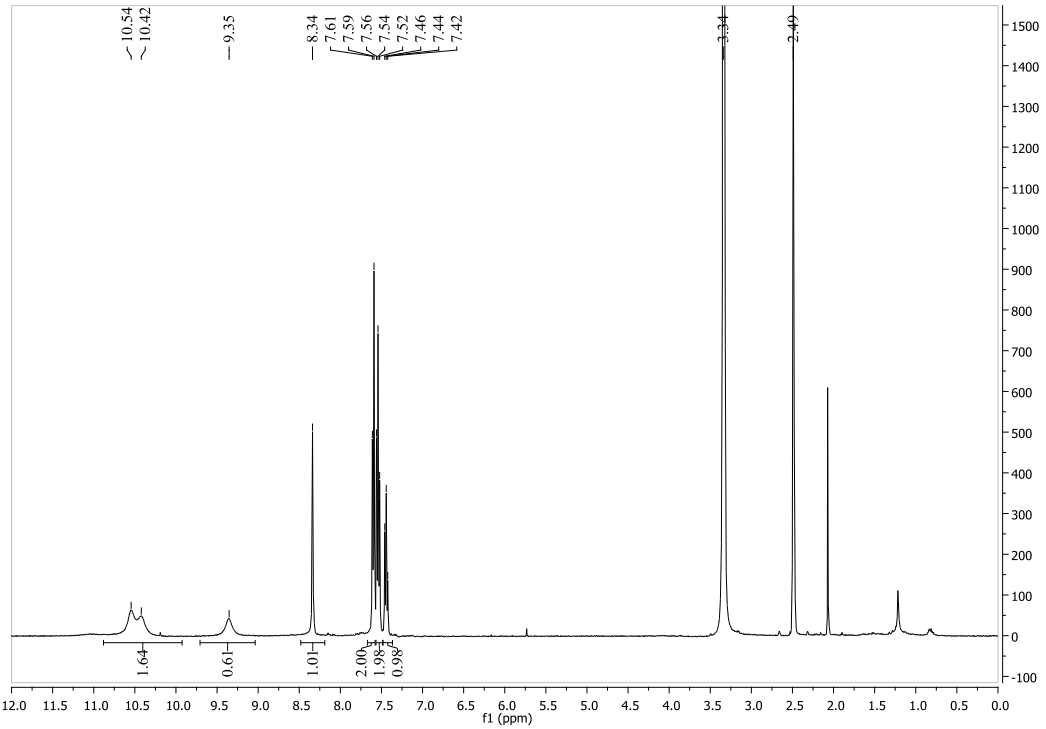
^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.6 and 10.4 (br d, 1.5H, OH or NH), 9.36 (br s, 0.5H, OH or NH), 8.34 (s, 1H, H-2), 7.70 – 7.57 (m, 2H, H-11), 7.54 (t, J = 7.7 Hz, 2H, H-12), 7.44 (t, J = 7.3 Hz, 1H, C-13).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 161.8 (C-8 or C-15), 151.2 (C-8 or C-15), 150.3 (C-2), 150.1 (C-4 or C-6), 138.4 (C-4 or C-6), 132.6 (C-10), 129.0 (C-12), 128.0 (C-13), 126.4 (C-11), 106.8 (C-5).

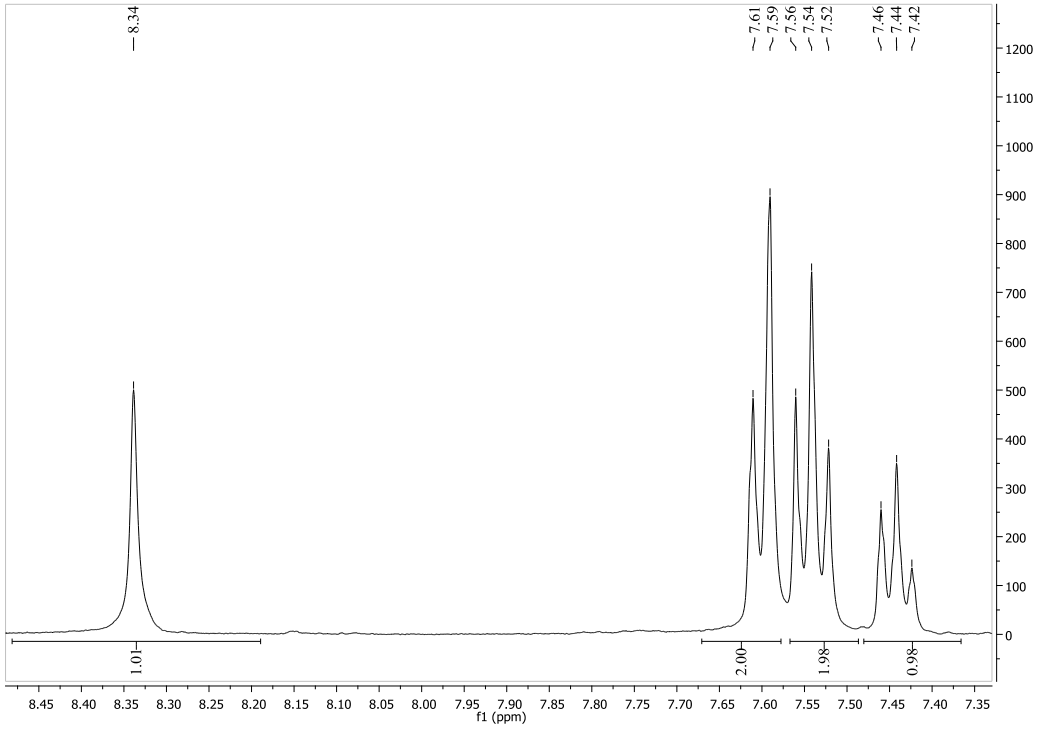
MS EI *m/z* (rel. %) 256 (28), 255 (40, M^+), 228 (50), 227 (100).

HR-MS Found 255.0765, calculated for $C_{12}H_9N_5O_2$ 255.0756.

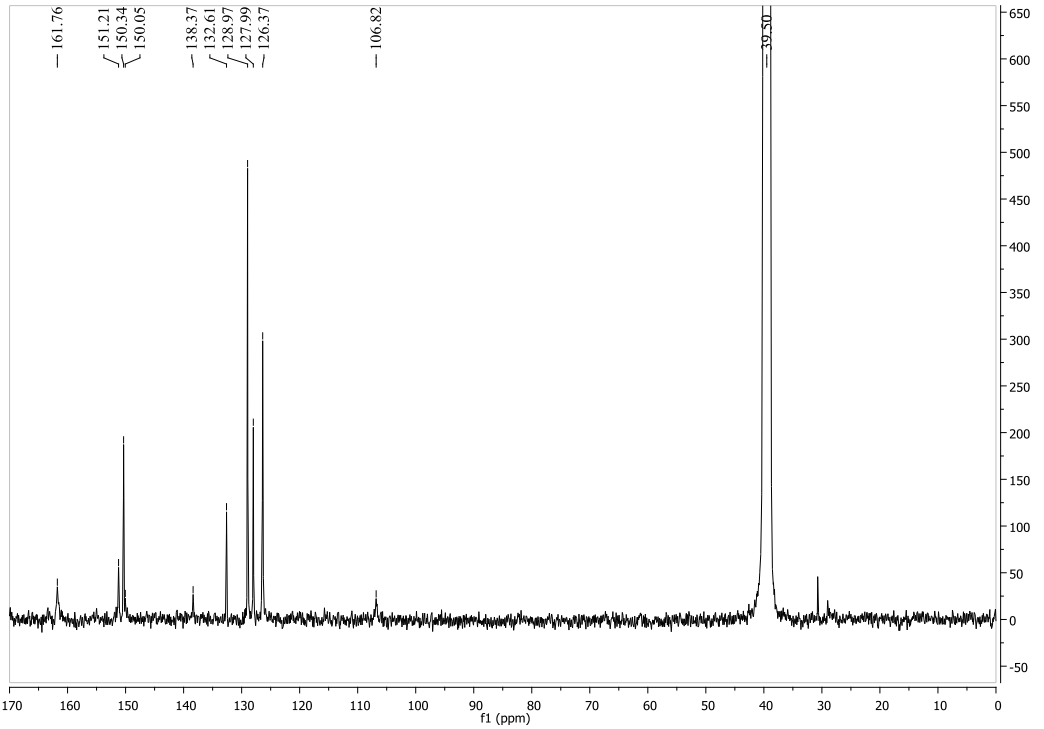
M.p. not obtained.



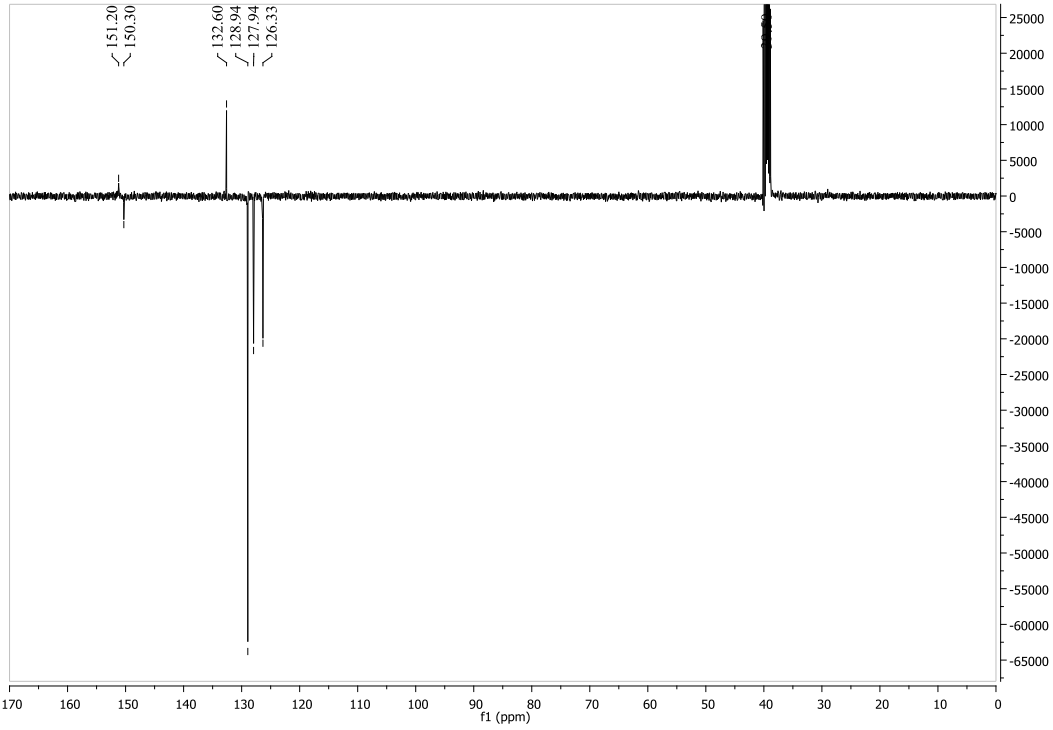
Spectrum 171. [1]H NMR of Unknown By-product (**48d**).



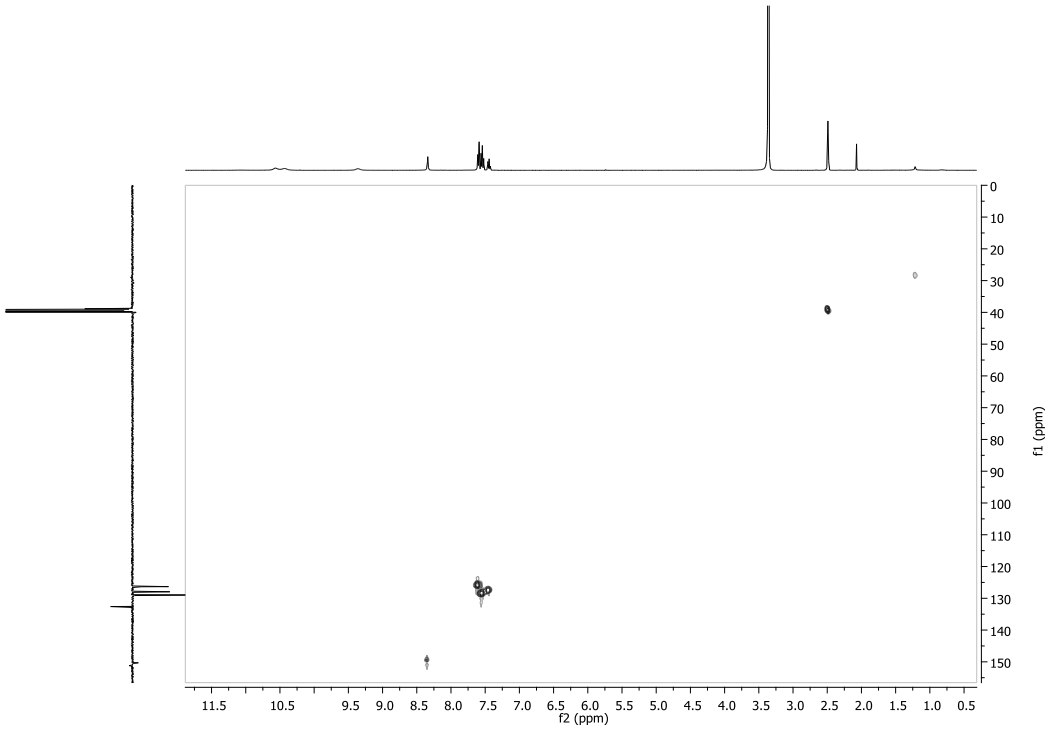
Spectrum 172. [1]H NMR of Unknown By-product (**48d**), expansion of the aromatic region.



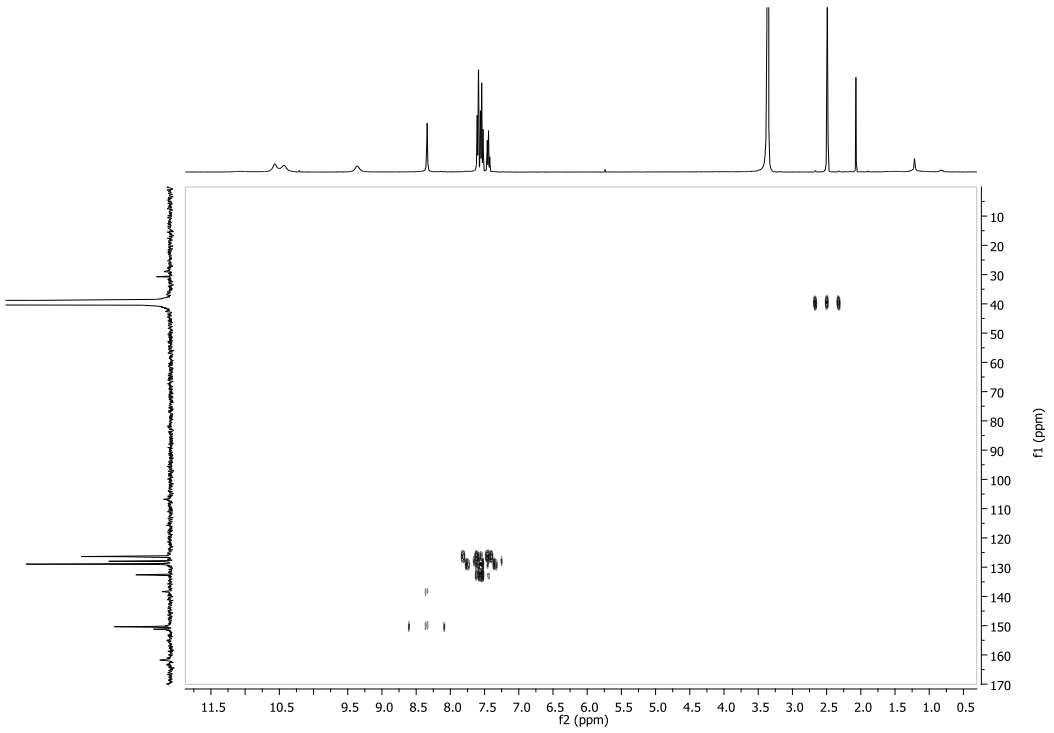
Spectrum 173. ^{13}C NMR of Unknown By-product (**48d**).



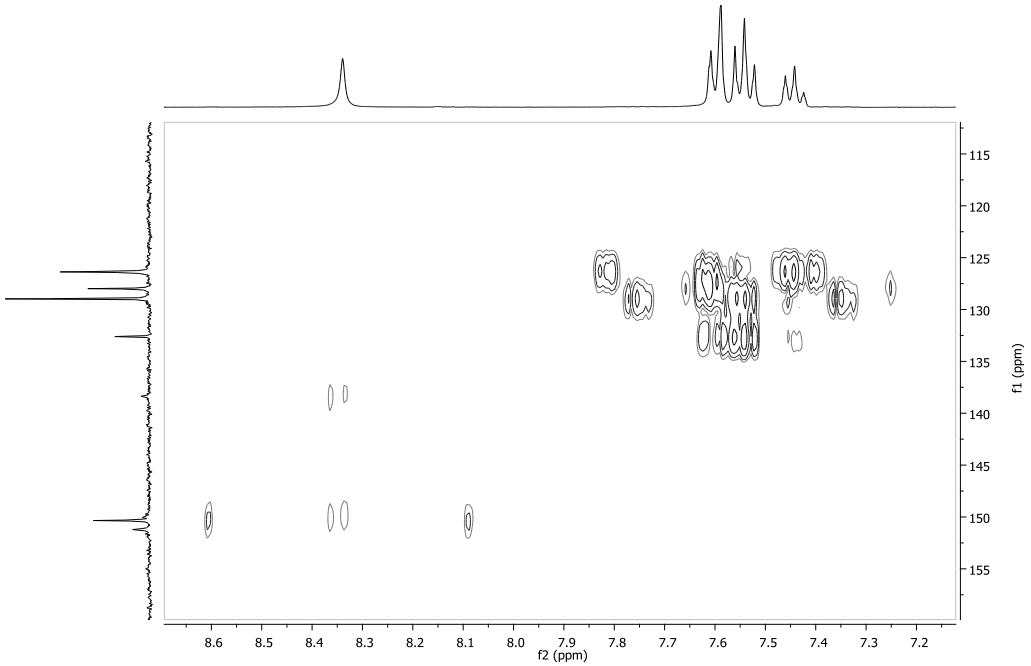
Spectrum 174. ^{13}C APT NMR of Unknown By-product (**48d**).



Spectrum 175. HSQC of Unknown By-product (**48d**).

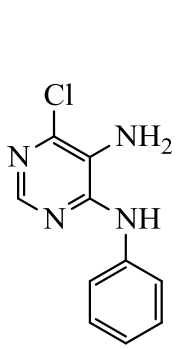


Spectrum 176. HMBC of Unknown By-product (**48d**).

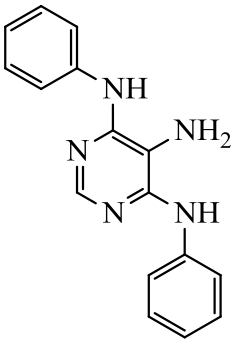


Spectrum 177. HMBC of Unknown By-product (**48d**), expansion of the aromatic region.

6-Chloro-N^4-phenylpyrimidine-4,5-diamine (10a) and N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (10b)



10a



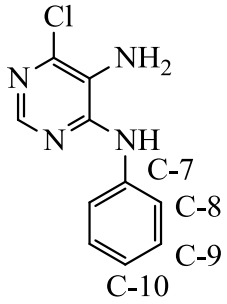
10b

Method 1: A mixture of 4,6-dichloro-5-amino-pyrimidine (**55**) (333 mg, 2.03 mmol), 6 mL distilled water, 1 mL EtOH, and 0.1 mL conc. HCl was stirred. Aniline (0.24 mL, 2.60 mmol) was added. The reaction mixture was refluxed for 14 h, then 5 mL of boiling water added and as the mixture cooled, a beige powder precipitated out of the mixture. The precipitate was collected and recrystallized in 2:1 methanol:water to give **10a** as pale orange needles (285 mg, 69%).

Method 2: A mixture of 4,6-dichloro-5-amino-pyrimidine (**55**) (251 mg, 1.53 mmol), aniline (0.18 mL, 2.00 mmol), triethylamine (0.295 mL, 2.10 mmol) and *n*-butanol (5 mL) was refluxed for 3 days. The solvent was removed *in vacuo* and the residue was purified using flash chromatography (hexanes – 1:4 ethyl acetate:hexanes – 1:2 ethyl acetate:hexanes) to give **10a** as an off-white powder (243 mg, 68%).

Method 3: 4,6-Dichloro-5-amino-pyrimidine (**55**) was heated at 130 °C with stirring in 0.95 mL aniline for 3 h. The mixture was concentrated *in vacuo* and purified using flash chromatography (hexanes with gradient to 100% ethyl acetate, then second flash 0-6% methanol in dichloromethane) giving N^4,N^6-diphenylpyrimidine-4,5,6-triamine (**10b**) (14 mg, pure and 70 mg, impure) and its hydrochloric salt (350 mg, impure).

6-Chloro-N^4-phenylpyrimidine-4,5-diamine (10a)



10a

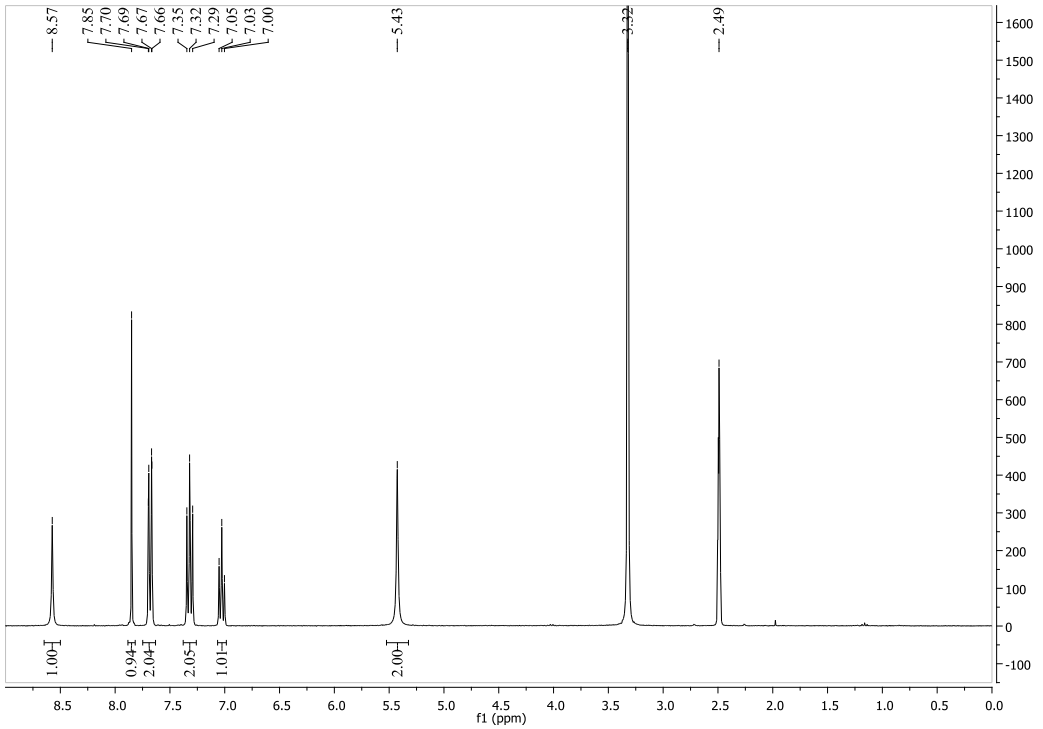
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.57 (br s, 1H, NH), 7.85 (s, 1H, H-2), 7.70 – 7.67 (m, 2H, H-8), 7.35 – 7.29 (m, 2H, H-9), 7.05 – 7.00 (m, 1H, H-10).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 148.8 (C-4 or C-6), 144.7 (C-2), 139.7 (C-7), 138.5 (C-4 or C-6), 128.4 (C-9), 124.8 (C-5), 122.7 (C-10), 120.5 (C-8).

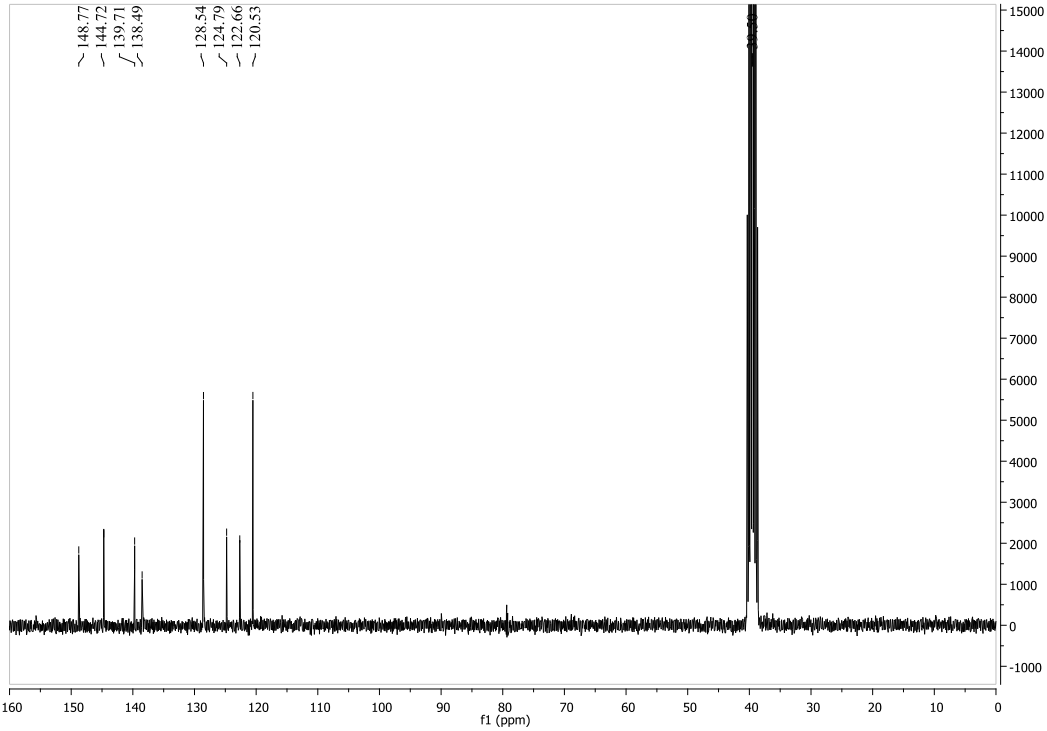
MS EI *m/z* (rel. %) 222/220 (32/100, M^+), 221/219 (41/85), 185 (11), 117 (10), 104 (31), 90 (12), 77 (25).

HR-MS Found 220.0509, calculated for $C_{10}H_9ClN_4$ 220.0516.

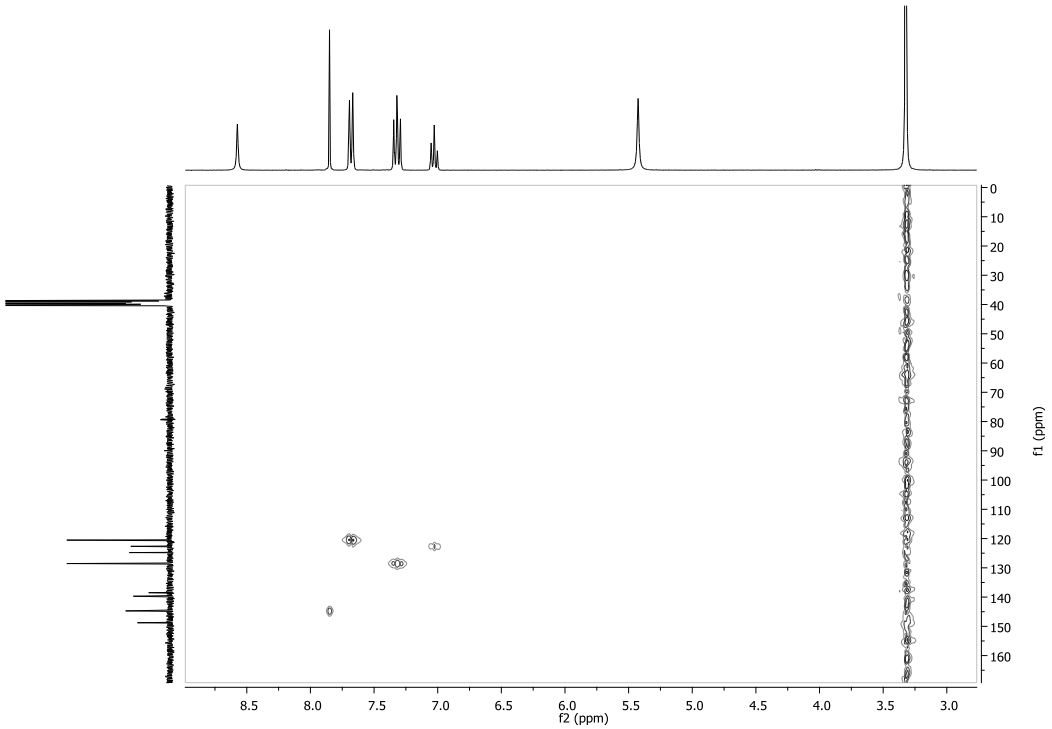
M.p. 175 °C (lit.[52] = 175-176 °C).



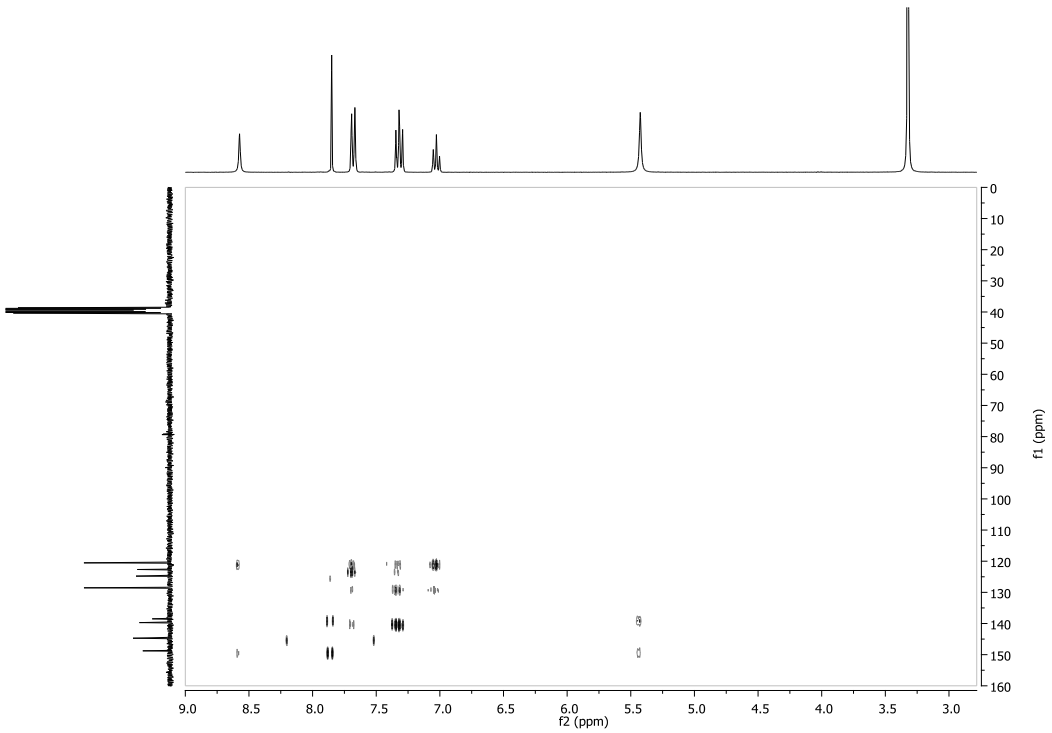
Spectrum 178. ^{1}H NMR of 6-Chloro-*N*^{4}-phenylpyrimidine-4,5-diamine (**10a**).



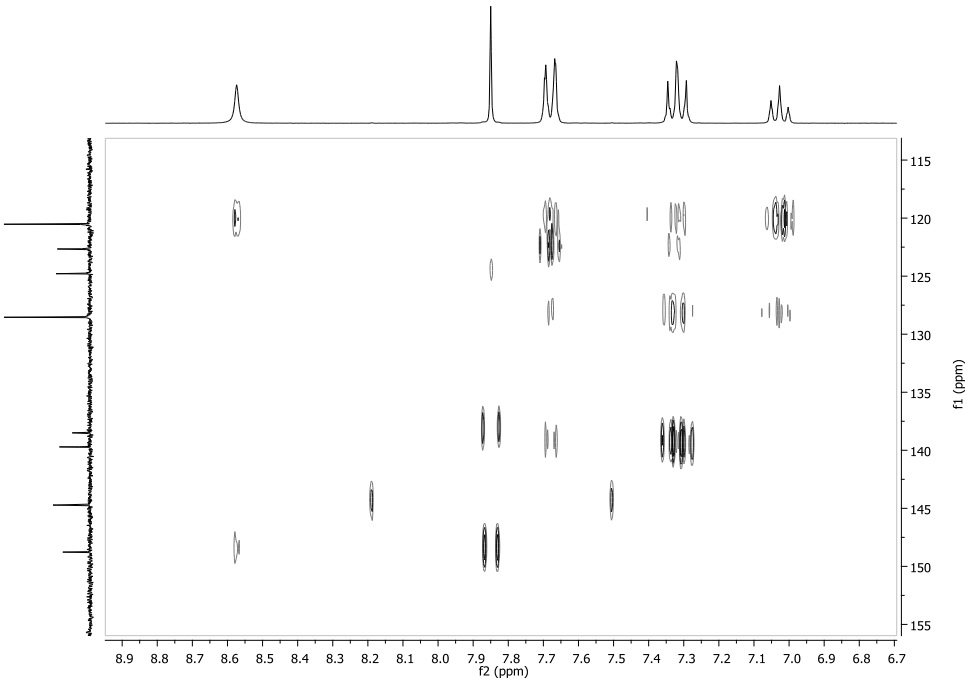
Spectrum 179. ^{13}C NMR of 6-Chloro- *N*^{4}-phenylpyrimidine-4,5-diamine (**10a**).



Spectrum 180. HMQC of 6-Chloro-N^4-phenylpyrimidine-4,5-diamine (**10a**).

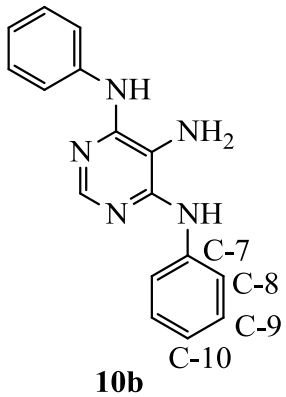


Spectrum 181. HMBC of 6-Chloro-N^4-phenylpyrimidine-4,5-diamine (**10a**).



Spectrum 182. HMBC of 6-Chloro-N^4-phenylpyrimidine-4,5-diamine (**10a**), expansion of the aromatic region.

N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (10b)



10b

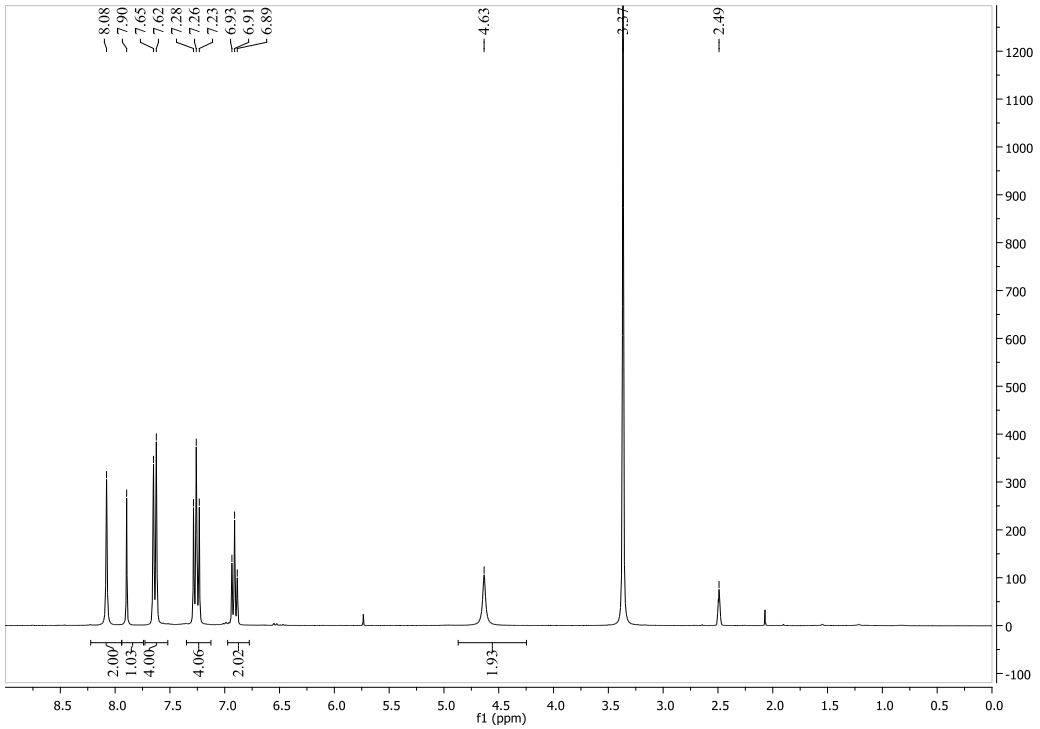
^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.08 (s, 2H, NH_2), 7.90 (s, 1H, CH), 7.64 (d, *J* = 7.7 Hz, 4H, H-8), 7.26 (t, J = 7.9 Hz, 4H, H-9), 6.91 (t, *J* = 7.3 Hz, 2H, H-10), 4.63 (br s, 2H, NH).

^{13}C NMR (DMSO-d_6, 75 MHz) δ 148.0 (C-4 and C-6), 146.0 (C-2), 141.2 (C-7), 128.4 (C-9), 121.0 (C-10), 119.4 (C-8), 110.8 (C-5).

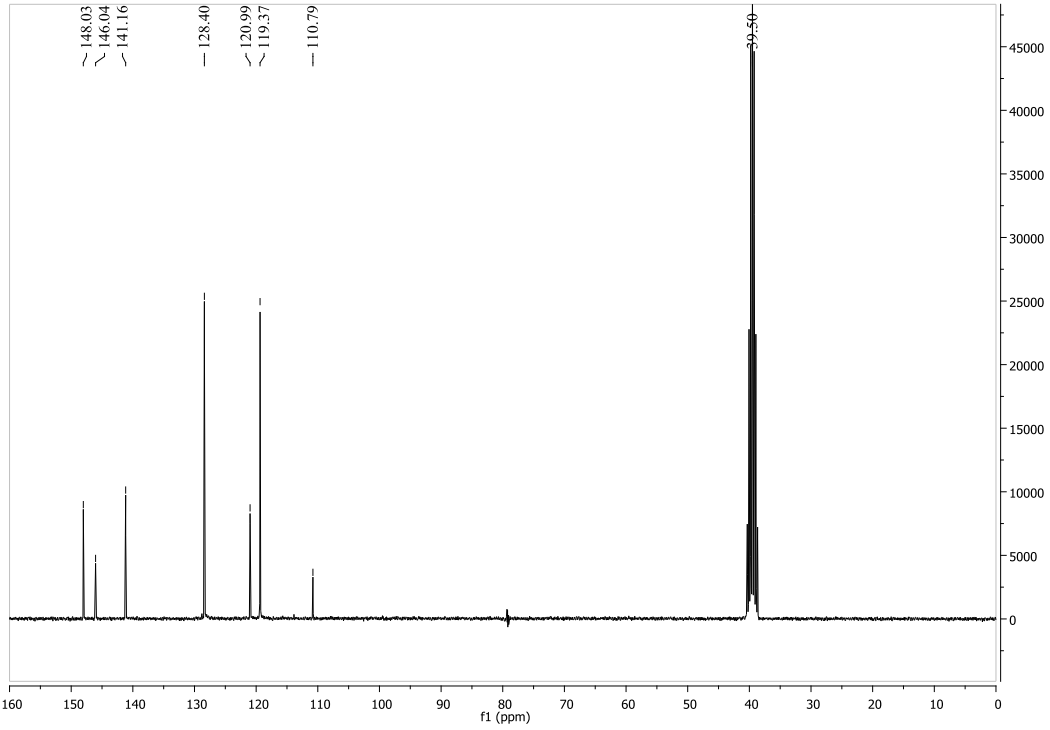
MS EI *m/z* (rel. %) 277 (100, M^+), 146 (6), 119 (7), 104 (9), 77 (15), 71 (7).

HR-MS Found 277.1317, calculated for $C_{16}H_{15}N_5$ 277.1327.

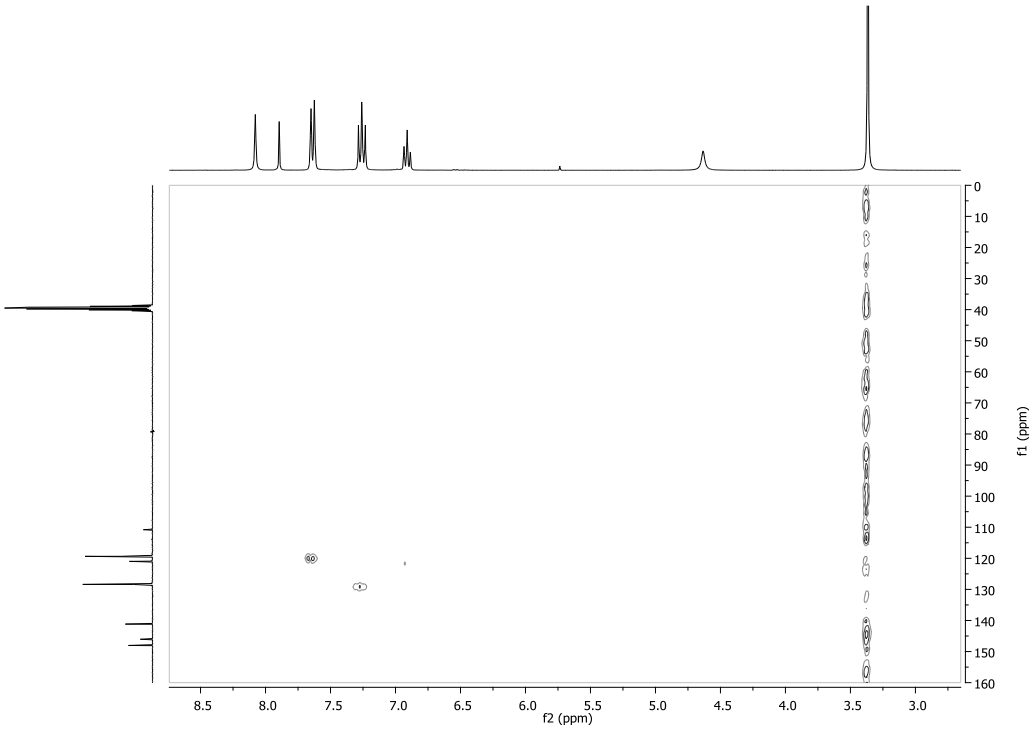
M.p. not obtained.



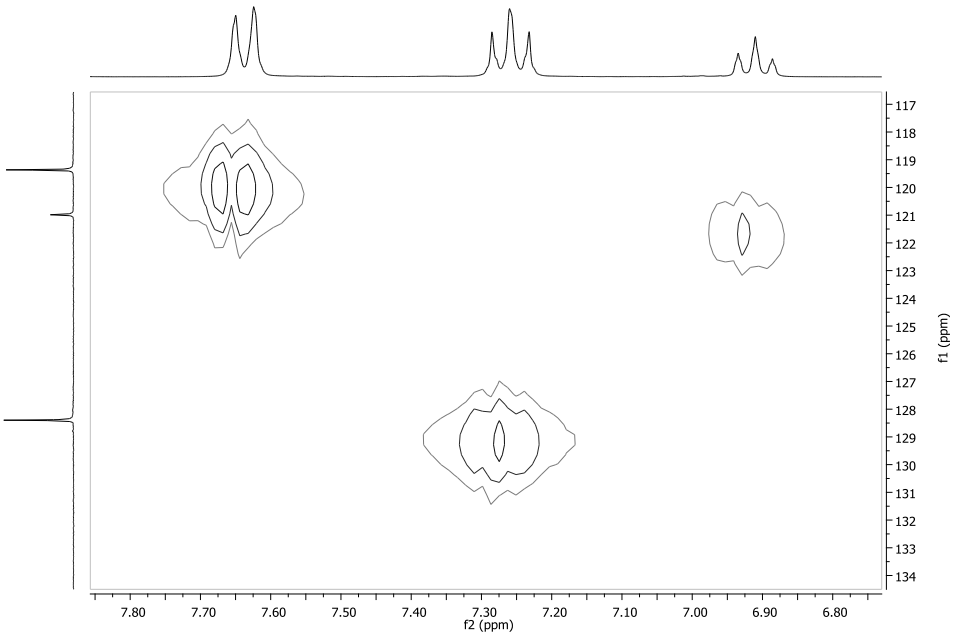
Spectrum 183. ^{1}H NMR of N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (**10b**).



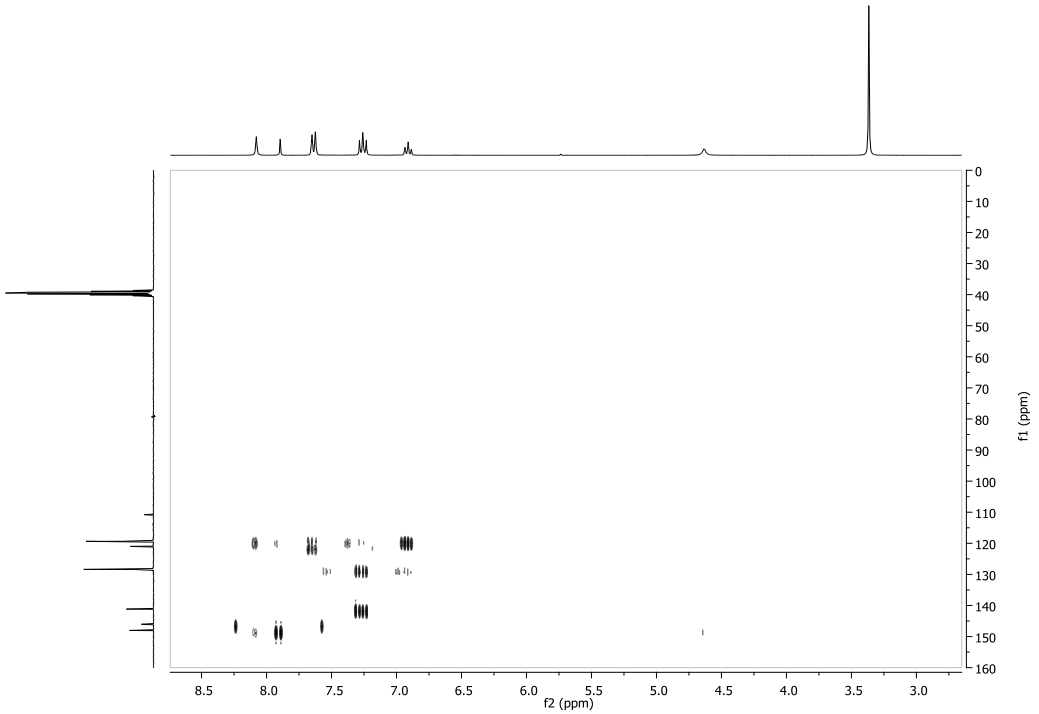
Spectrum 184. ^{13}C NMR of N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (**10b**).



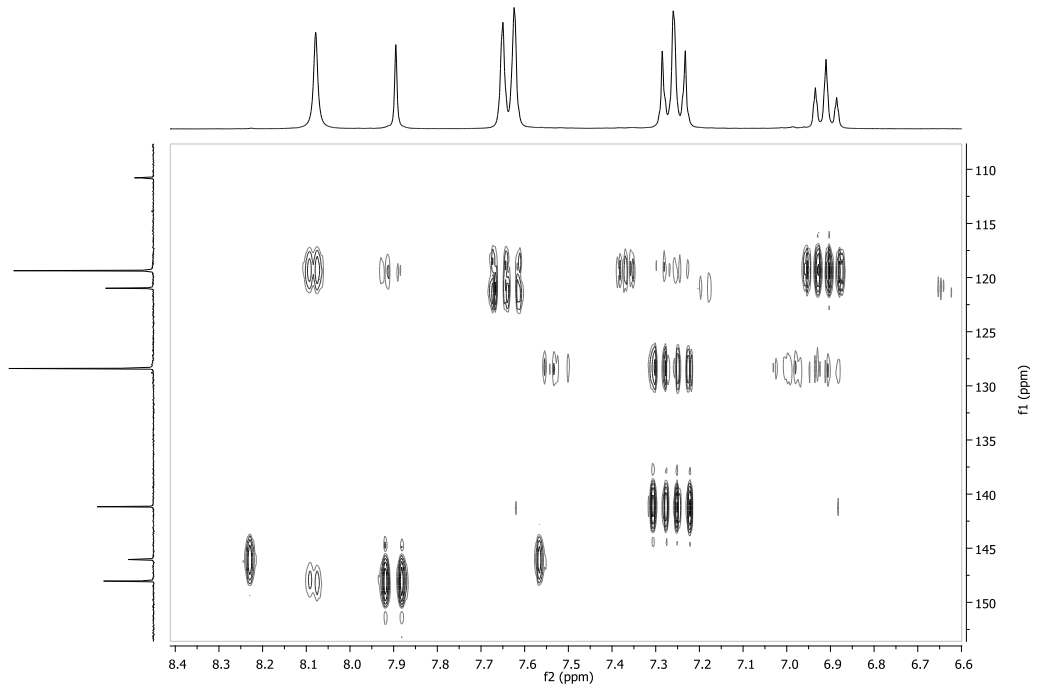
Spectrum 185. HMQC of N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (**10b**).



Spectrum 186. HMQC of N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (**10b**), expansion of the phenyl region.

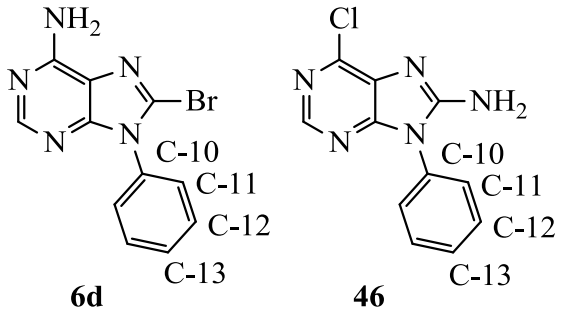


Spectrum 187. HMQC of N^4,N^6-Diphenylpyrimidine-4,5,6-triamine (**10b**).



Spectrum 188. HMQC of N^4,N^6-diphenylpyrimidine-4,5,6-triamine (**10b**), expansion of the aromatic region.

Mixture of products from amination of 8-bromo-6-chloro-9-phenylpurine (6d and 46)



6d **46**

8-Bromo-6-chloro-9-phenylpurine (120 mg, 0.388 mmol) was heated in concentrated ammonium hydroxide solution in a sealed vial at 100 ˚C for 17 h. The ammonium hydroxide was removed *in vacuo* and the residue purified *via* flash chromatography (0 – 10% methanol in dichloromethane) to give 50 mg of a 1:1 mixture of **6d** and **46**. Suggested assignments for NMR data are included below.

8-Bromo-9-phenyl-*9H*-purin-6-amine (6d)

^{1}H NMR (DMSO-d_6, 600 MHz) δ, 8.06 (s, 1H, H-2), 7.67 – 7.50 (m, 5H, H-11 to H-13), 7.47 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.9 (C-6), 153.30 (C-2), 151.8 (C-4), 133.8 (C-10), 129.4 (C-13), 129.3 (C-11 or C-12), 128.1 (C-11 or C-12), 126.40 (C-8), 118.9 (C-5).

MS ESI 290.0 $(M+H)^+$.

HR-MS Found 288.9963, calculated for $C_{11}H_8N_5Br$ 288.9963.

6-Chloro-9-phenyl-*9H*-purin-8-amine (46)

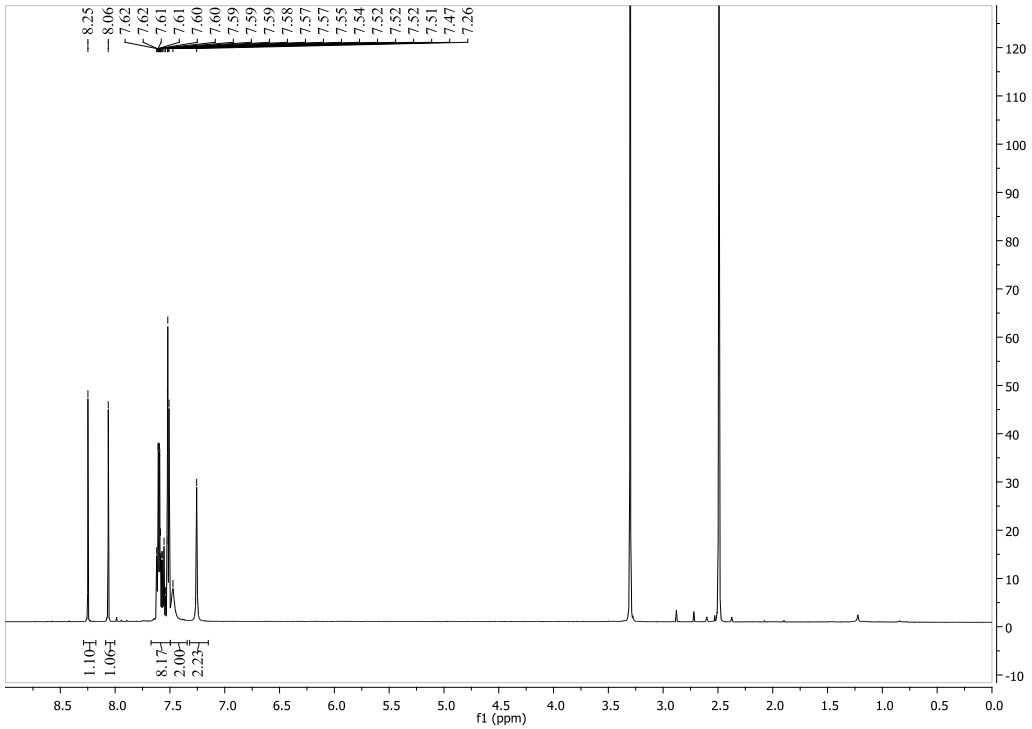
^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.25 (s, 1H, H-2), 7.67 – 7.50 (m, 5H, H-11 to H-13), 7.26 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.0 (C-8), 147.5 (C-2), 131.6 (C-5), 140.2 (C-4 or C-6), 153.8 (C-4 or C-6), 132.4 (C-10), 129.7 (C-11 or C-12), 129.2 (C-13), 127.4 (C-11 or C-12).

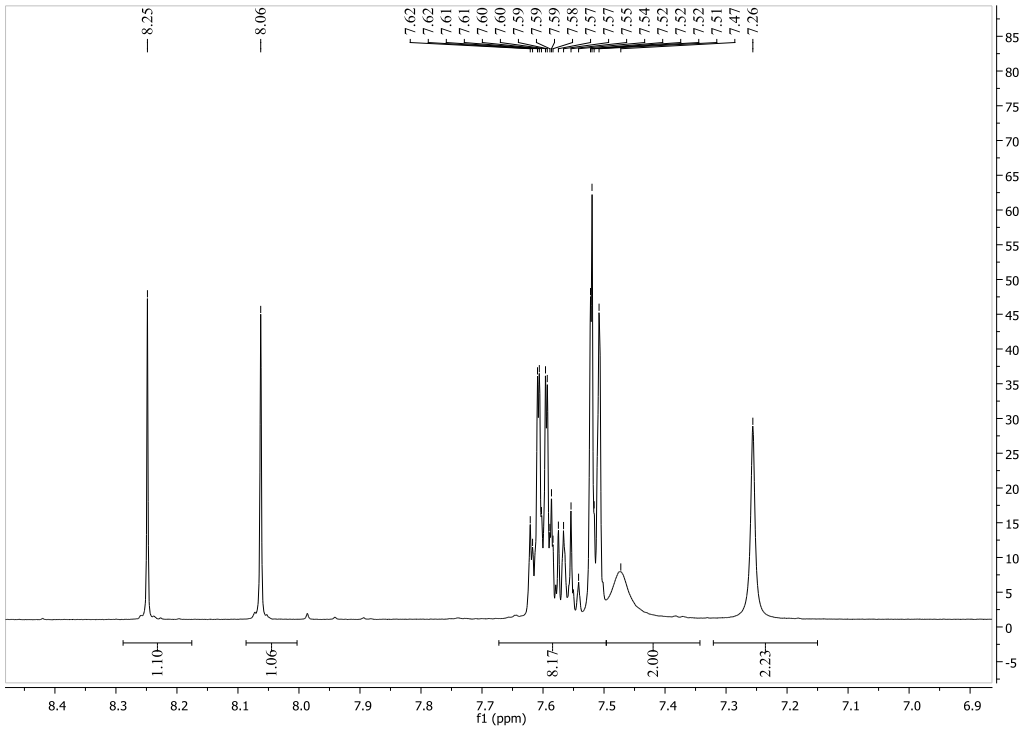
MS ESI 246.0 $(M+H)^+$.

HR-MS Found 245.0474, calculated for $C_{11}H_8N_5Cl$ 277.1327.

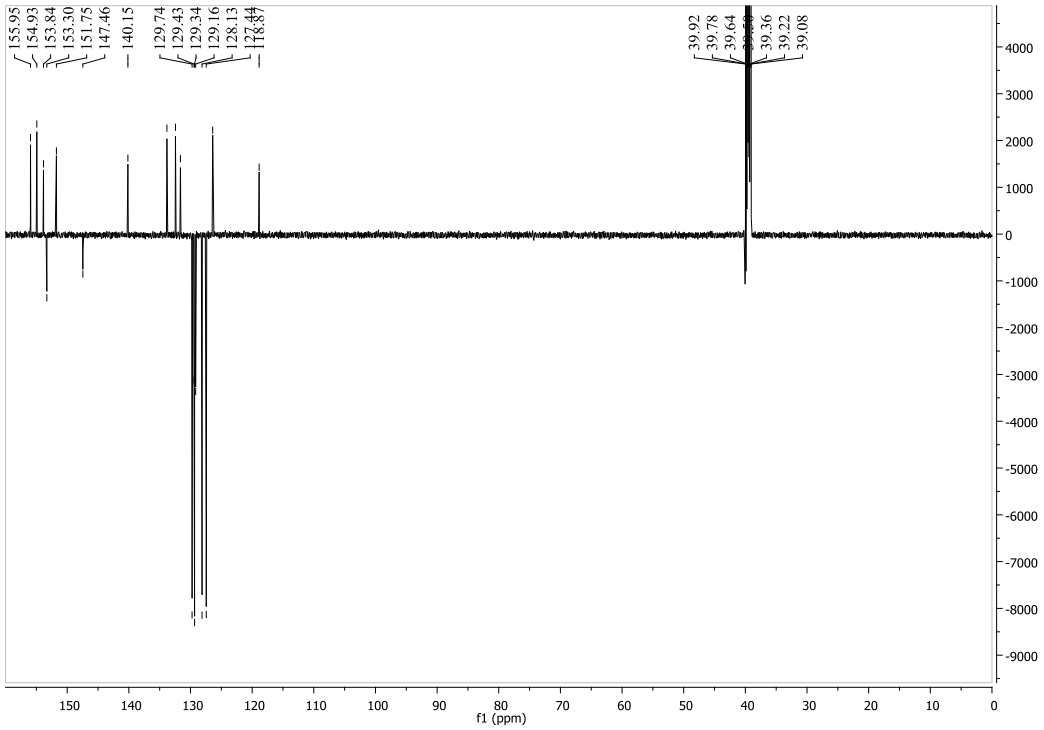
MS EI *m/z* (rel. %) for the mixture: 291/289 (98/100, M^+ for **6d**), 247/245 (78/23, M^+ for **46**), 210 (32), 183 (34), 156 (14), 104 (7), 77 (38).



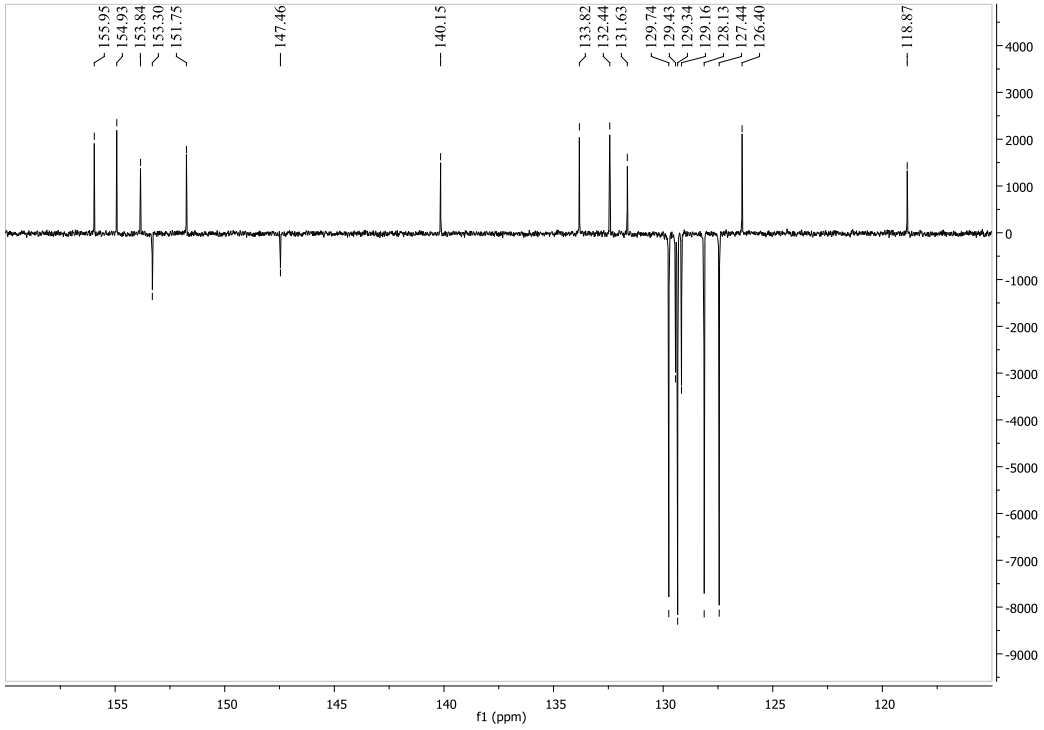
Spectrum 189. [1]H NMR of the mixture of 8-bromo-9-phenyl-*9H*-purin-6-amine (**6d**) and 6-chloro-9-phenyl-*9H*-purin-8-amine (**46**).



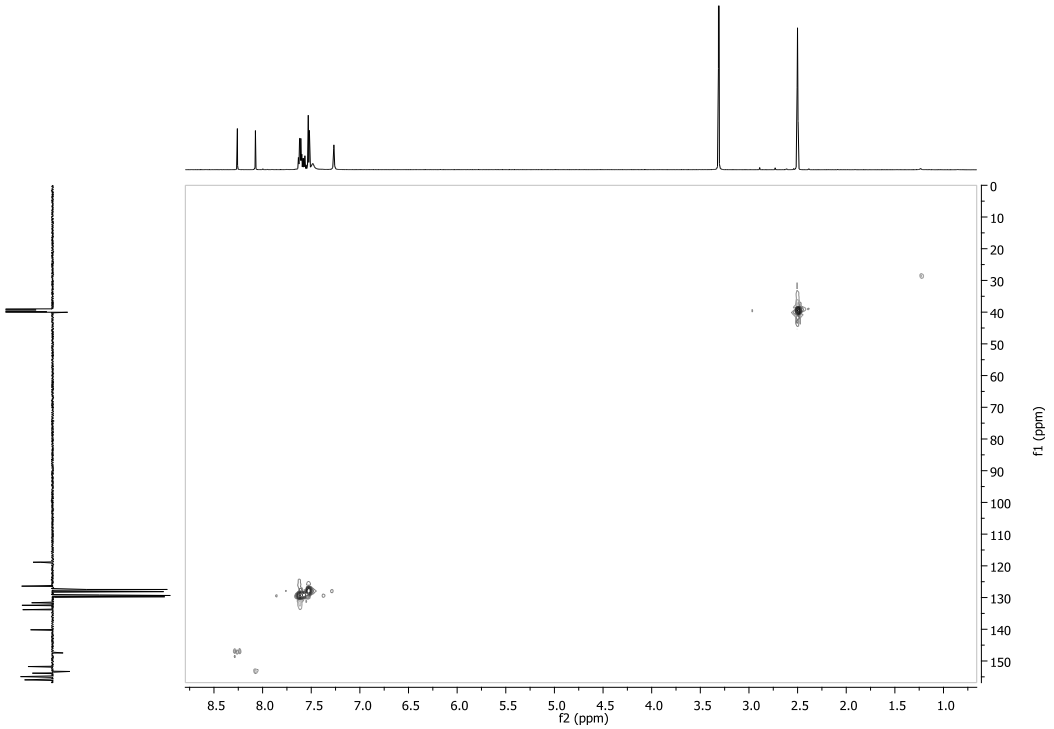
Spectrum 190. [1]H NMR of the mixture of **6d** and **46**, expansion of aromatic region.



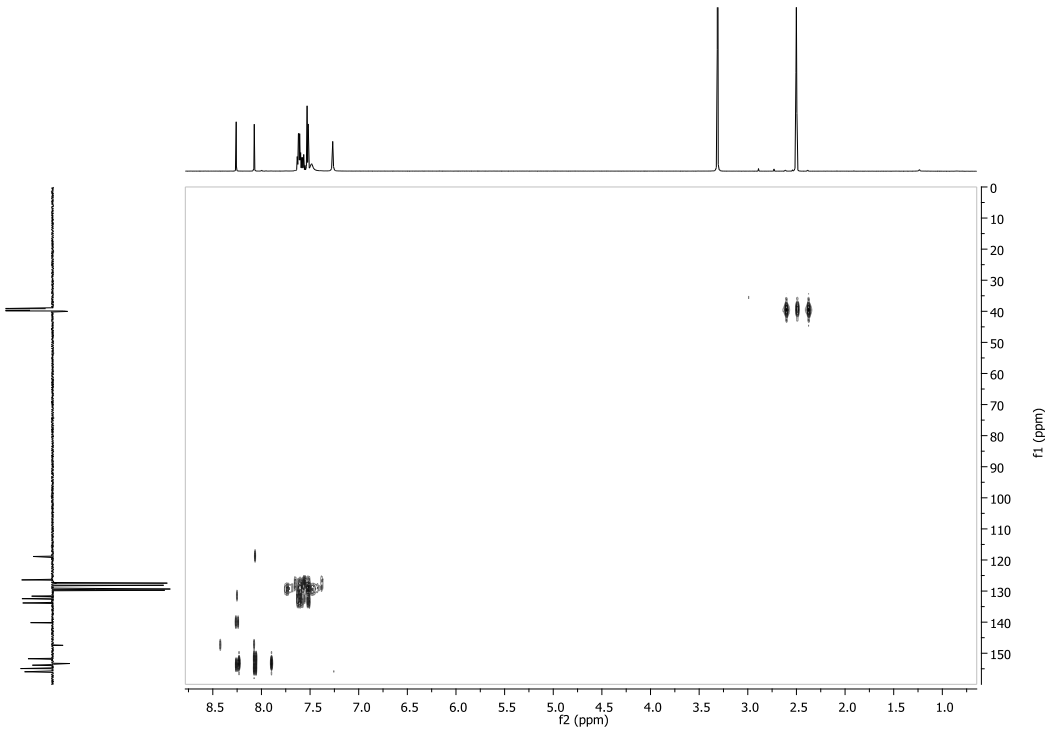
Spectrum 191. ^{13}C NMR of the mixture of **6d** and **46**.



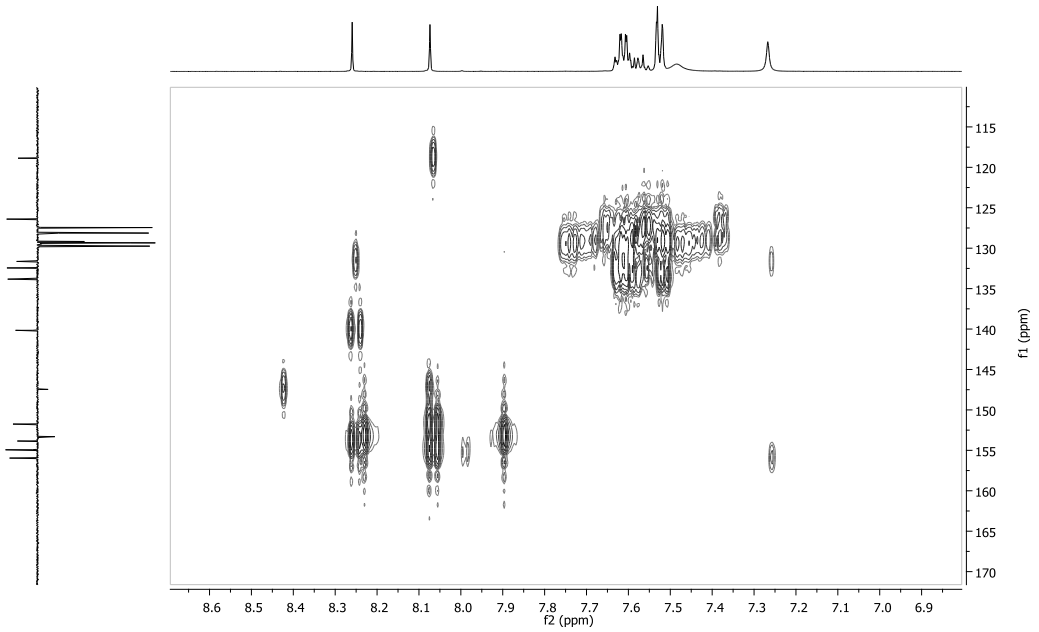
Spectrum 192. ^{13}C NMR of the mixture of **6d** and **46**, expansion of aromatic region.



Spectrum 193. HSQC of the mixture of **6d** and **46**.



Spectrum 194. HMBC of the mixture of **6d** and **46**.



Spectrum 195. HMBC of the mixture of **6d** and **46**, expansion of the aromatic region.

CHAPTER 8

8. APPENDICES

8.1. Appendix 1

Paper I:

NMR and X-ray Structural Studies on 3-Benzyl-8-bromoadenine.

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NMR and X-ray Structural Studies on 3-Benzyl-8-bromoadenine

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FPO

8-Bromoadenine was benzylated in the presence of base to give a mixture of two regioisomers. One was easily recognized as 9-benzyl-8-bromoadenine, but the other structure could not be determined with absolute certainty by NMR. Therefore, X-ray crystallography was used to prove that the benzyl group was attached to N-3. Furthermore, it is shown that the 3-benzyl adenine derivative exists as the amine tautomer both in the crystalline state as well as in solution (DMSO-d_6), with restricted rotation around the N^6—C6 bond.

AQ1

J. Heterocyclic Chem., **00**, 00 (2010).

INTRODUCTION

Generally, reaction of adenine with alkyl halides under neutral conditions gives the 3-alkylated product as the major isomer. However, selective N-9 alkylation is often seen when the reaction is performed in the presence of a base [1]. The numbering of the adenine system is shown in Figure 1. When 8-bromoadenine is alkylated under basic conditions, the selectivity for the 9-position is generally reduced, and mixtures of the 3- and 9-alkylated isomers are often reported [2–6]. The isomeric distribution is qualitatively the same in Cu-mediated N-arylation of 8-bromoadenine [7]. When 8-bromoadenine was benzylated in our laboratories under basic conditions, we also obtained two isomers, but we found that the position of the benzyl group in one of the isomers could not be determined with absolute certainty by NMR spectroscopy. Furthermore, the ^{1}H-NMR spectrum of the same compound showed some intriguing features (discussed below) and, as a result, we performed thorough NMR and X-ray structural studies on this compound.

F1

RESULTS AND DISCUSSION

8-Bromoadenine (**1**) [2] was reacted with benzyl bromide in the presence of K_2CO_2 at ambient temperature as shown in Scheme 1. The minor product was easily identified as the 9-benzylated isomer **2** based on comparison with data reported before [4] as well as ^{1}H-^{13}C HMQC and HMBC NMR experiments. Compound **2** is reported to be the major isomer when the benzylation was performed at higher temperature [4], but in our hands more than two products were formed at elevated temperatures and the purification of products was rather tedious. The spectral data for our major product were in good agreement with what has bee reported for compound **3** before. However, despite the fact previous literature structure elucidation is claimed to be based on ^{1}H-^{13}C HMQC and HMBC NMR (no details given) [4], we found our HMBC data to be inconclusive. The CH_2 protons correlate to two carbon shifts; the C-2 at 143.9 ppm and a peak at 149.8 (quaternary C). Unfortunately, it was not possible to determine if the latter peak was the C-4 or C-6 shift, and hence, it was not possible to establish if the benzyl group was situated at N-3 or N-1. Both the peak at 149.9 and a second peak (quaternary C) at 153.6 correlated to H-2 and neither of them correlated with the NH_2 in the HMBC spectrum.

S1

Because there are several reports on the formation of N-3 alkylated 8-bromoadenine, where the structure elucidation is reported to be based on ^{1}H-^{13}C HMQC and HMBC NMR [4,5,7], which in fact may not be conclusive, we decided to determine with absolute certainty whether the major product formed was the isomer **3** or **4**. For these purposes, we turned to X-ray crystallography. Before this investigation, crystal structures of ten 8-bromoadenines were available in the Cambridge Structural Database (CSD, Version 5.31 of November 2009 [8]). All these molecules carry an additional ethyl group or a sugar moiety, which is always attached to N-9 as for isomer **2**.

The result of the X-ray structural investigation shown in Figure 1 confirms that isomer **3** has been crystallized. This is not only the first ever X-ray structure of an N-3 functionalized 8-bromoadenine but in fact also the first N-3 functionalized adenine where neither N-7 nor N-9 act as ligands for metal ions or carry additional

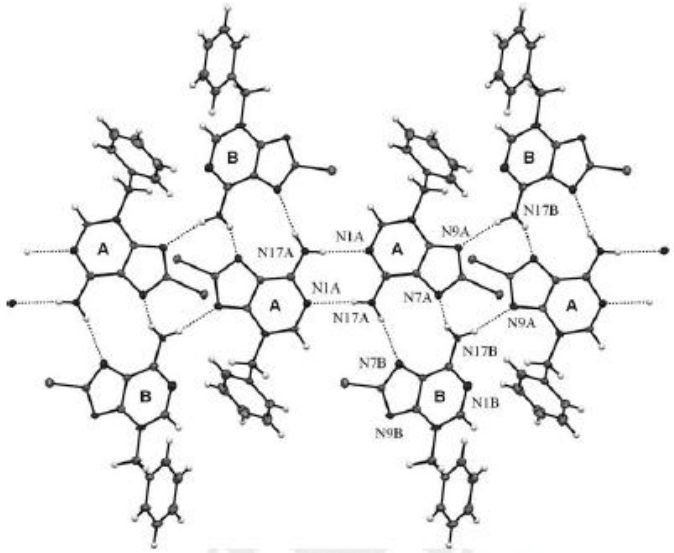

Figure 2. Adenine molecules connected by hydrogen bonds into one-dimensional chains or tapes. The indicated H···N distances are in the range 2.09(4) – 2.16(3) Å. It can be seen that molecule **A** participates in a larger number of strong interactions as the aromatic N atoms accept a total of three H atoms compared with only one H atom for molecule **B**.

selectivity is higher when the reaction is conducted at ambient temperature compared with elevated temperatures. Furthermore, it is shown that the 3-benzyl adenine derivative exists as the amine tautomer both in the crystalline state as well as in solution (DMSO-d_6), but with restricted rotation around the N^6—C6 bond.

EXPERIMENTAL

The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument and the decoupled ^{13}C-NMR spectra were recorded at 75 MHz using instrument mentioned above. The ^{1}H-^{15}N HSQC spectrum was recorded with a Bruker AVII600 instrument (pulse program hsqcetf3gpsi using sensitivity improvement). All NMR spectra were obtained in DMSO-d_6. Mass spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionizing voltage, and are presented as *m/z* (% relative int.). Melting points were determined with a Büchi Melting Point B-545 apparatus and are uncorrected. DMF was obtained from a solvent purification system, MB SPS-800 from MBraun. Silica gel for flash chromatography was purchased from Merck, Darmstadt, Germany (Merck No. 09385). All other reagents were commercially available and used as received. AQ3

9-Benzyl-8-bromo-9H-purin-6-amine (2) and 3-benzyl-8-bromo-3H-purin-6-amine (3). A mixture of 8-bromoadenine [2] (220 mg, 1.03 mmol), DMF (5 mL) and potassium carbonate (280 mg, 2.03 mmol) was stirred at ambient temperature under N_2 atmosphere for 30 min. Benzyl bromide (0.18 mL, 1.5 mmol) was added and the mixture stirred for another 4 h. The mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with 0–5% MeOH in CH_2Cl_2 to give compounds **2** (45 mg, 15%) and **3** (128 mg, 42%).

9-Benzyl-8-bromo-9H-purin-6-amine (2). Colorless powdery crystals, m.p. 238°C (Lit. [4], 226–227°C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.16 (s, 1H, H-2), 7.47 (br s, 2H, NH_2),

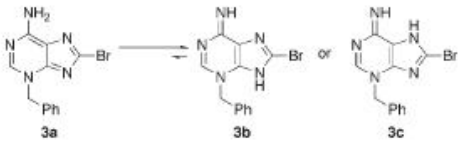

Figure 3. Possible tautomers of compound **3** in solution.

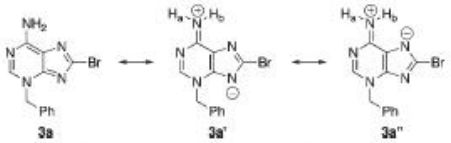

Figure 4. Resonance forms **3a'** and or **3a''** that may contribute to the structure of tautomer **3a**.

7.33–7.21 (m, 5H, Ph), 5.35 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 154.7 (C-6), 153.0 (C-2), 150.9 (C-4), 135.9 (C-8), 128.7 (Ph), 127.8 (Ph), 127.1 (Ph), 126.5 (Ph), 119.0 (C-5), 46.6 (CH_2); ms: *m/z* 305/303 (26/26, M^+), 91 (100).

3-Benzyl-8-bromo-3H-purin-6-amine (3). Colorless powdery crystals, m.p. 233–235°C (Lit. [4], 239–240.5°C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.53 (s, 1H, H-2), 8.22 (br s, 1H, NH_2), 8.10 (br s, 1H, NH_2), 7.40–7.29 (m, 5H, Ph), 5.46 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 153.6 (C-6), 149.8 (C-4), 144.0 (C-2), 139.3 (C-8), 135.8 (Ph), 128.7 (Ph), 128.1 (Ph), 127.8 (Ph), 121.5 (C-5), 52.0 (CH_2); ms: *m/z* 305/303 (28/28, M^+), 91 (100).

X-ray crystallographic analysis for compound 3. Crystals of **3** suitable for X-ray crystallography were obtained from a solution of compound **3** in acetonitrile placed inside a larger vial containing ethyl acetate. They are unstable at room temperature due to loss of co-crystallized ethyl acetate solvent molecules, and X-ray data collection with Apex-2 [11] was thus performed at 105 K. Apex II single crystal CCD-diffractometer, MoK_α radiation ($\lambda = 0.71069$ Å), 0.30 mm × 0.30 mm × 0.26 mm block-shaped specimen, data integration and cell refinement with SAINT-Plus [12], absorption correction by SADABS [13], structure solution by and least-squares refinement on F^2 with SHELXTL [14]. Solvent molecules are located on two different inversion centers, each with a maximum allowed occupancy of 0.500 and form distinct channels running through the crystal along the *ab*-diagonal. The geometries of independent solvent molecules were constrained to be similar within a standard deviation of 0.002 Å for bond lengths and 0.003 Å for 1–3 distances.

3-Benzyl-8-bromo-3*H*-purin-6-amine ethyl acetate solvate: $C_{12}H_{10}BrN_5{\cdot}0.5C_4H_8O_2$, M = 348.21, triclinic, $P-1$, a = 8.4937(6) Å, b = 12.9096(9) Å, c = 15.2238(10) Å, α = 101.723(1)°, β = 105.162(1)°, γ = 108.192(1)°, $Z = 4$, $N_{observed}$ = 5830, $R[F^2 > 2\sigma(F^2)] = 0.058$, $wR(F^2) = 0.146$, CCDC 786213.

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8.2. Appendix 2

Viruses for which the four target molecules, 3- and 9-benzyl-8-bromoadenine and 3-benzyl-8-oxoadenine were tested against:

- anti-feline corona virus (FIPV) and anti-feline herpes virus activity and cytotoxicity in CRFK cell cultures;
- antiviral activity and cytotoxicity in: HEL cells, HeLa cells, and Vero cells;
- anti-influenza virus activity and cytotoxicity in: MDCK cells;
- anti-HIV activity and cytotoxicity in: MT-4 cells;
- against cytomegalovirus in human embryonic lung (HEL) cells; and
- against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells.

CHAPTER 9

9. REFERENCES

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